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AGEING TROPICAL FISH BY GROWTH RINGS IN THE OTOLITHS

Prepared by

J. Gjøsaeter, P. Dayaratne, O.A. Bergstad,
H. Gjøsaeter, M.I. Sousa, I.M. Beck

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Sardinella longiceps	-	H. Gjøsaeter
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Other species	-	H. Gjøsaeter

I.M. Beck participated during the development of practical methods and by the reading of otoliths.

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AUTHORS' ADDRESSES

O.A. Bergstad, J. Gjøsaeter : Department of Fishery Biology
University of Bergen
N-5011 Bergen-Nordnes
NORWAY

P. Dayaratne : N.A.R.A.
Crow Island
Colombo 15
SRI LANKA

I.M. Beck, H. Gjøsaeter : Institute of Marine Research
N-5011 Bergen-Nordnes
NORWAY

M.I. Sousa : Instituto de desenvolvimento pesqueiro
Av. Mao Tse Tung No. 250
Maputo
MOZAMBIQUE

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ABSTRACT

The report reviews information on primary growth rings in otoliths, the methods used to prepare them for counting, and their use in ageing fish. Much emphasis is placed on testing the reliability of the ageing method.

Reference is made to work on species from 29 different families, including many tropical species. The paper contains case studies on Sardinellas, Decapterus russellii, Hilsa kelee, Stolephorus sp., Nemipterus japonicus, Epinnula sp., tropical Myctophidae and Dussumieria acuta.

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1. INTRODUCTION

Estimating age forms an essential part of the assessment and management of a fish stock and is necessary to study both growth and mortality.

Age can be estimated by statistical approaches based on analysis of the number of modes in a length frequency distribution or on the progression of modes in a time series of such distributions. Another approach is to count the periodic markings in otoliths, scales or bones. Both these methods are usually more easily applied to temperate than to tropical fishes.

As stated by Parrish (1979) the problem of age determination of tropical species has until recently "been the source of more complaint than effort". Much of the effort put into the field was reviewed by Brothers (1979) who concludes that ageing by means of micro-structures in the otoliths seemed to be the most promising method available at the moment.

It was discovered, in the early seventies, that the otoliths of some tropical and temperate fishes contained primary growth increments which seemed to be formed daily (Pannella 1971, 1974). This appeared to form a basis for obtaining potentially very accurate age estimates in a reasonably simple way.

The list of species found to have primary otolith increments has steadily grown. Since species from very different environments and geographical regions are included, it seems reasonable to assume that incremental growth is a universal phenomenon in fish otoliths.

For some species there are, however, indications that considering the primary growth rings as daily marks may be too simplistic. Therefore, it seems necessary to verify the reliability of the method for each new species and probably also for different environments.

The present report reviews information of primary growth rings in otoliths, the methods used to prepare them for counting, and their use in ageing the fishes. Much emphasis is placed on how to approach the problem of testing the reliability of the ageing method. Following the general parts a few case studies which may serve as illustration of the principles involved.

2. REVIEW

Structure

Studies of the ultrastructure of fish otoliths have revealed alternating patterns of incremental and discontinuous zones arranged concentrically around a nucleus. (Degens, Denser and Haedrich, 1969, Dunkelberger, Dean and Watabe, 1980, Pannella 1974, Tanaka, Mugiya and Yamada, 1981 and others).

The incremental zones consist of aggregates of needle-like crystals of calcium carbonate oriented perpendicular to the outer margin of the otolith. Between the crystals is a protein matrix called the intercrystalline matrix. There is a sheet-like organic matrix in the discontinous zone (interlamellar matrix).

Under the light microscope these structures appear as narrow dark lines and usually broader light lines. Using the scanning electron microscope, the discontinuous zones of etched otoliths usually appear as narrow grooves between bands of fractured crystals.

Increment number vs age.

Table 2.1. provides a list of fish species for which various kinds of studies of primary otolith structure or growth have been reported. (A complete list of references follows the case studies of Ch. 4). At least two major accounts do not appear in Table 2.1, namely the reviews by Brothers (1979) and Pannella (1980). Brothers lists an extensive

Table 2.1. List of fish species for which studies of primary growth of otoliths have been reported. Species, age range covered, origin of material and general methodology are indicated. Main results appear when the study was designed as a "verification study". See last section for complete list of references. S-large scale experiment (plastic bags, basins, ponds), L-lab. experiment, N-natural population, LM-light microscopy, SEM-scanning electron microscopy, TEM-transmission electron microscopy, A-acetate replica technique (and LM).

	Age	Area	Material	Prep. Reading	Results of verification attempts			Verification method	Authors
					Periodicity of main patterns	Other patterns	Notes		
Clupeidae									
Clupea harengus	0-135d	temp	S	LM	daily			known age	Gjøsæter, 1981; Gjøsæter & Øiestad, 1981
C.harengus	larvae/juv	temp	N	LM	assumes daily			mode progr.	Townsend & Graham, 1981
C.harengus	larvae/juv	temp	L,S	LM	variable		no.of rings dependent on growth rate	known age	Geffen, 1982
C.harengus	larvae/juv	temp	L,N	LM	lower than daily in early larval period			known age	Lough et al., 1982
Sardinella longiceps	90-160d	trop	N	L					Gjøsæter, 1981
S. sp.	juv/adults	trop	N	LM	probably daily			following one population	Dayaratne, 1983
Sardina pilchardus	larvae	temp	N	LM					Ré, 1983
Engraulidae									
Engraulis mordax	6-100d	sub.t	L,N	LM,A SEM	daily			known age	Brothers, Mathews and Lasker, 1976
E.mordax	0-25d	sub.t	N	LM					Methot & Kramer, 1979
Stolephorus purpureus	larvae/juv	trop	N,S	LM	daily			following one population	Struhsaker & Uchiyama, 1976

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Other patterns	Notes	Verification method	Authors
Stolephorus sp.	juv.	trop	N	LM	?		Two types of rings observed		Gjøsæter, 1981
Anguillidae									
Anguilla anguilla	Juv. and adults	temp	S	A,SEM					Liew, 1974
Salmonidae									
Oncorhyncus nerka	0-100d	temp	L	LM	daily above 10°C	weekly sub.daily	no rings when t<5°C	known age	Marshall & Parker, 1982
O.nerka	-28d juv.	temp temp	S L	LM LM	daily			known age known age	Wilson & Larkin, 1980, 1982
O.tshawytscha	juv.	temp	L	SEM,LM	daily		dependent on feeding frequency,	known age	Neilson & Geen, 1982
O.kisutch	juv.	temp	L						Campana, 1983
Salmo gairdneri	juv.	temp.	L	LM(UV)	Daily	3d pattern induced by feeding manip.	indep. of feeding freq. and 32 d starvation	marking	Campana, 1983
Osmeridae									
Osmerus eperlanus	adult	sub.arc.	N	SEM	probably daily in summer	weekly Bimonthly		weekly and seasonal bands	Timola, 1977
Mallotus villosus	larvae adults juv.	sub.arc.	S,N	LM	mostly daily in juveniles		less than 1 per day in adults	known age comp.growth Back-calc. birthdates	Gjøsæter & Monstad, 1982

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Other patterns	Notes	Verification method	Authors
Gadidae									
Gadus morhua	larvae	temp	N	LM	inconcl.			morph.staging	Gjøsæter & Tilseth, 1982
G.morhua	juv.	temp	N	A	daily in juv.			counting rings between cyclical marks	Pannella, 1971
G.morhua	<1 yr	temp	N	LM	?			Back calculated spawning season	Steffensen, 1981
G.morhua	larvae	temp	L,N	LM,SEM	daily			known age of larvae	Radtke & Waiwood, 1980
G.morhua	larvae, juv.	temp.	S,L	LM	variable in larvae daily in juv.		differences between otolith types	known age	Bergstad, 1983
G.morhua	larvae	temp.	L	TEM					Dale, 1983
Micromesistius poutassou	juv.	temp.	N	LM,A	?				Gjøsæter, Beck and Monstad, 1979
"	juv.	sub. arct. temp.	N	SEM	probably daily			comp.age dv. from litt.data	Townsend & Shaw, 1982
Urophysis chuss	adults	temp	N	A	daily in young	monthly		counting rings between cyclical marks	Pannella, 1971
Merlucciidae									
Merluccius angustimanus	juv. (1-2 yr)	temp (sub.t)	N	LM/A?	daily			counting rings between annuli	Brothers, Mathews and Lasker, 1976
M.bilineares	adult	temp	N	A	daily in young	weekly, forthnight-		counting between other cy-	Pannella, 1971

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Results of verification attempts: Other patterns	Results of verification attempts: Notes	Verification method	Authors
M.paradoxus	adult	sub.t.	N	SEM	daily in young	weekly & monthly + spawning checks		"	Morales-Nin, 1980
Merluccius sp.	< 7 yr	temp	N	LM,A, SEM	daily			counting between annuli	Brothers, Mathews and Lasker, 1976
M.productus	larvae	temp.	L	LM	daily		narrow age range	known age	Bailey, 1982
Cyprinodontidae									
Fundulus heteroclitus	embryo/ larvae/ juv.	temp	L	SEM,LM	daily		no effect of growth variation within range investigated	known age	Radtke & Dean, 1982
Atherinidae									
Leuresthes tenuis	< 26 d	temp	L	LM,A, SEM	daily			known age	Brothers, Mathews and Lasker, 1976
Menidia menidia	< 70 d	temp	N,L	LM	daily			known age	Barkman, 1978
"	juv.	"	N	LM					Barkman, Bengtson and Beck, 1981
Holocentridae									
Holocentrus rufus	< 4 yr	trop	N	A	daily in young			counting betw. other cycl. marks	Panella, 1974
Scorpaenidae									
Sebastes melanostromus	larvae/ juv	temp	N	LM					Moser and Ahlstrom, 1978
Sebastes sp.	larvae	sub.arct.	N,L	LM,SEM	daily			known age	Radtke, 1980

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Results of verification attempts: Other patterns	Results of verification attempts: Notes	Verification method	Authors
Sebastes sp.	larvae	sub.arct.		N	LM daily	probably		comp.growth backcal.extrusion time	Peney & Anderson, 1981
S.diploroa	prejuv.	temp	N,L	LM					Boehlert, 1981
Centropomidae									
Centropomus undecimalis	< 4 yr	trop	N	A	daily?	monthly		counting betw. other cycl. marks	Panella, 1974
Percichthyidae									
Morone saxatilis	< 15 mts.	temp	N	LM,A, SEM	daily			backcalc. sp. dates comp. growth	Brothers, Mathews and Lasker, 1976
Centrarchidae									
Micropterus salmoides	< 1 yr	temp	N	LM	daily			following one cohort	Schmidt & Fabrizio, 1980
Carangidae									
Vomer Setapinnis	< 4 yr	trop	N	A	daily			counting betw. other cycl.m.	Pannella, 1974
Lutjanidae									
Lutjanis apodus	< 4 yr	trop	N	A	daily	monthly		"	Pannella, 1974
Pomadasyidae									
Haemulon sciurus	adults	trop	N	A	daily	monthly		"	Pannella, 1974
H.flavolineatum	< 100d.	trop	N	LM					Brothers & McFarland, 1981

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Other patterns	Notes	Verification method	Authors
Sciaenidae									
Pseudootolithus elongatus	adults	sub. trop	N	A	daily in juveniles			seasonal bands	Le Guen, 1976
P.typus P.senegalensis	adults	sub. trop	N	A	daily in juveniles			"	Le Guen, 1976
Cynoscion janaicensis	adults	trop	N	A	daily	monthly		counting betw. other cycl.m.	Pannella, 1974
Micropogon furnieri	adults	trop	N	A	daily			counting betw. cycl.marks	Pannella, 1974
Plagioscion monti	< 250d	trop	N	LM					Worthmann, 1980
Chaetodontidae									
Chaetodon miliaris	adults	trop	N	LM	daily ?			Comp.growth	Ralston, 1976
Cichlidae									
Tilapia nilotica	juv.	temp	L	SEM	daily			known age studying formation mechanism	Tanaka, Mugiya and Yamada, 1981
T.guineensis	adults	trop	N	LM	daily ?	weekly ?		assumes daily and weekly	Fagade, 1980
T.Mossambica	<60d	temp	L	LM	daily			known age	Taubert & Coble, 1977
Lepomis gibbosus L.cyanellus L.macrochirus	<176d	temp	L	LM	daily		dependent on body growth as manip. by temp.	known age	Taubert & Coble, 1977

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Results of verification attempts: Other patterns	Results of verification attempts: Notes	Verification method	Authors
Gobidae									
Clevelandiaios Ilypnus gilberti Quietulay-cauda	Post larvae	temp	N	LM	probably daily			comp.length of larval period	Brothers, Mathews and Lasker, 1976
Scaridae									
Sparisoma aurofrenatum	adults	tropic	N	LM	probably daily	spawning marks			Miranda, 1981
Channichtyidae									
Champsocephalus gunnari	adults	antar-	N	LM	?		counting problems		Kochkin, 1982
Scombridae									
Thunnus albacares	adults	trop	N	LM,A	daily			Tetracycline marks	Wild & Foreman, 1980
T.albacares	adults	trop	N,S	LM	daily			Holding exp. comp.growth	Uchiyama & Struhsaker, 1981
Katsuwonus pelamis	adults	trop	N	A	less than daily		Possibly caused by stress due to maturation	Tetracycline marks	Wild & Foreman, 1980
K.pelamis	adults	trop	N,L	LM	daily			comp.growth	Uchiyama & Struhsaker, 1981
Scomber scombrus	larvae	temp	N	LM					Kendall & Gordon, 1979
Istiophoridae									
Makaira nigricans	adults	sub. trop	N	SEM	?				Radtke, Collins and Dean, 1982

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Results of verification attempts: Other patterns	Results of verification attempts: Notes	Verification method	Authors
Pleuronectidae									
Parophrys vetulus	larvae	temp	L,N	LM	probably daily		Lab. results ambiguous	known age	Laroche, Richardson and Rosenberg, 1982
"	juv.	temp	N	LM					Rosenberg and Laroche, 1982
"	juv	temp	N	LM					Rosenberg, 1982
Platichthys stellatus	juv.	temp	L	LM,SEM	average daily		variable rates although unaff. by feeding freq.	Tetracycline marks	Campana, 1983
Platichthys stellatus	juveniles	temp	N,L	LM,SEM	daily	subdaily	unaff. by temp. fluct. or changes of photoperiod	Tetracycline marks	Campana & Neilson, 1982
Pleuronectes platessa	adults	temp	N	SEM	daily rings not observed			counting within seasonal marks	Blacker, 1975
Scophthalmus maximus	larvae	temp	L	LM	variable		no.of rings dependent on growth rates	known age	Geffen, 1982
"	larvae	temp.	S	LM					Rosenberg & Haugen, 1982
Labridae									
Thalassoma bifasciatum Halichoeres bivittatus	juv. & adult	trop	N	LM	daily			Marking otoliths through handling and feeding	Victor, 1982
Gonostomatidae									
Maurolicus muelleri	larvae adults	temp	N	SEM,LM	daily in juveniles		possibly lower in adults		Gjøsæter, 1981

Table 2.1. (cont'nd).

	Age	Area	Material	Prep. Reading	Results of verification attempts: Periodicity of main patterns	Results of verification attempts: Other patterns	Results of verification attempts: Notes	Verification method	Authors
Cyprinidae									
Carassius auratus	juv.	temp	L	SEM	daily			Studying formation mechanism	Mugiya, Watabe and Yamada, 1981
Semotilus corporalis	larvae, juv.	temp	L,N	LM	daily			known age	Victor & Brothers, 1982
Bagridae									
Chrysichthys Nigrodigitatis	adults	trop	N	LM	possibly daily in young	possibly weekly		Counting rings between annuli	Fagade, 1980
Myctophidae									
Stenobrachius leucopsarus	-60d	temp	N	LM					Methot, 1981
Benthosema pterotum B.fibulatum	larvae-adult	trop	N	LM,SEM					Gjøsæter, 1978, 1981,
Symbolophorus evermanni	adult	trop	N	LM,SEM			rings easy to read		Gjøsæter, 1981
Myctophum spinosum	adult	trop	N	LM			rings easy to read		Gjøsæter, 1981
Diaphus diademophilus	adult	trop	N	LM			rings difficult to read		Gjøsæter, 1981
Lampanyctus sp.	adult	trop	N	LM,SEM			rings difficult to read		Gjøsæter, 1981

series of families in which he has found members having primary increments supposedly representing daily growth.

Not all studies, referred to in Table 2.1 were originally designed for the purpose of testing whether or not the observed increments could safely be regarded as daily growth units. In some, solely morphological descriptions were provided, while in others growth or age structure analysis was performed, based on the assumed direct correspondence between ring counts and age.

We would, however, focus some attention on results of "verification studies". The general approach to the problem is indicated under the heading 'verification method' (a rather thorough description of the different approaches will follow in Ch. 3). Undoubtedly, some will find our selection of "verification studies" inadequate. Some would probably include those we rejected, while others would be even more selective than we found reasonable. Our sole intention, however, is to provide a general overview of previous results in order to, if possible, draw some general conclusions.

Now, Table 2.2 is a summary of conclusions to "verification studies". 64 papers were considered in Table 2.1, dealing with a total of 79 species. Of these, we decided that 51 species were studied with the aim of verifying the presence of daily growth zones (note, some species were studied several times in separate papers, while some papers report results for several species.....). For 31 of these 51 species, there seems to be a rather firm basis for concluding that the increments were on the average formed daily in the age interval studied. Possibly one may add another 12 species for which the results are promising, although less firmly based. Certainly, this strongly supports the original suggestion by Pannella (1971, 1974) and others that daily growth units in otoliths is a phenomenon common to most fishes from a wide variety of environments.

Table 2.2. General conclusions drawn from studies aimed at verifying the presence of daily increment formation on otoliths. Summary of Table 2.1.

General conclusion	No. of Species				
	Total	Sub-trop.	Trop.	Temp.	Arctic/ Antarct.
Inconclusive	1				1
Possibly daily	12	3	5	2	2
Daily in period investigated	31	3	11	17	
Significant deviation from daily pattern*	7	1		5	1

* not including cases where the deviation was caused by starvation.

Now, this is not to say that all fishes always and everywhere show a number of increments on their otoliths directly corresponding to age. Since our knowledge of the underlying mechanisms of cyclic increment formation is at most limited (see below), it is impossible to pinpoint the cases where severe deviations can be expected to occur. It seems rather clear, however, as noted by Brothers (1974), Pannella (1971, 1980) and others, that increment deposition becomes more irregular as age increases. In many temperate species indications point to slowing down or interruption of ring formation in late juveniles or adults (Pannella, 1971; Timola, 1977; Gjøsæter and Monstad, 1982).

Then there are the rather obvious cases of no otolith growth (or sometimes otolith resorption) during periods of severe stress or actual starvation (Pannella, 1980). Wild and Foreman (1980) found that the rate of increment formation decreased during gonad maturation of Skipjack tuna (Katsuwonus pelamis). Also, experimental evidence for interruptions induced by low temperature have been reported (Taubert and Coble, 1975; Marshall and Parker, 1982), probably meaning that no increments are formed during winter in many temperate species.

In only one case has the frequency of increment deposition been shown to be correlated with the growth rate of the fish, namely in the study of Atlantic herring (Clupea harengus L.) and turbot (Scophthalmus maximus) larvae by Geffen (1982). This may, however, not reflect the rarity of the phenomenon, but rather the fact that very few workers checked this possibility (note however, examples by Barkman (1978) and Radtke and Dean (1982)).

Now, many of these problems may not be relevant to tropical conditions, especially when working with fast growing species having rather short life-spans. Thus, the method may at present seem especially promising for tropical species.

Before proceeding any further, we will review briefly results of the more basic studies regarding the causative factors involved in cyclic increment formation.

Causal factors.

There is no general agreement as to the mechanisms underlying cyclic ring formation. Light, temperature and feeding periodicity have all been suggested as important factors. Taubert and Coble (1977) argued that an internal circadian rhythm entrained by the light/dark cycle, regulated the formation of daily rings in Tilapia mossambica. Marshall and Parker (1982) believe that photoperiod is the dominant factor of incremental otolith growth in Oncorhynchus nerka and that feeding rhythm is of little importance. Similar conclusions were reached by Tanaka, Mugiya and Yamada (1981) studying Tilapia nilotica, and Mugiya, Wataba and Yamada (1981) studying Carassius auratus. Conversely Neilson and Geen (1982) concluded that frequency of ring formation in Oncorhynchus tshawytscha was influenced by feeding frequency but not by photoperiod or temperature cycles.

Rannou and Thiriot-Quievreux (1975) have shown that Coryphaenoides guentheri living in deep waters with no light or other cyclical stimuli has an otolith structure similar to that of fish in shallow waters.

Brothers (1979) believed that water temperature was an important factor in the regulation of otolith ring formation, but this view is not supported by most other studies (e.g. Marshall and Parker 1982, Neilson and Geen 1982).

Pannella (1980) finds that the fishes or life stages showing well-defined diurnal activity cycles also have the most distinct daily growth increments on their otoliths. Thus, typical diurnal migrators or fishes showing diurnal rhythms in their feeding activity have better developed patterns than species showing rather continuous activity. This may indicate that physiological changes due to changes in activity levels may be involved.

3. METHODS

3.1 Introduction

Past studies of primary otolith growth can, at least superficially, be classified into three main categories (excluding now studies of strictly descriptive nature):

a) Studies of the basic processes involved in otolith growth; specifically the mechanisms responsible for the timing of increment formation.

b) Indirect attempts to verify that increments are deposited at an average rate of 1 ring$\cdot d^{-1}$, and thus that ring counts provide unbiased estimates of age.

c) Use of otoliths for age determination in studies of life history and population dynamics (i.e. growth and survival analysis).

As mentioned in the introduction, attempts to determine whether the increments can indeed be assumed to represent daily growth should be made prior to applying increment counts for routine ageing.

Accordingly, a discussion of useful approaches to such initial studies will form the main part of this chapter. Having determined the basic assumptions to be valid, the actual collection of age data is rather straight-forward.

It appears rather obvious that studies belonging to category (a) above are crucial for determining how reliable any age estimate based on otoliths really is. Still, we decided to concentrate on discussing the more indirect approaches. Our reason for doing so is that, to many users, the basic studies often prove too demanding as regards funds and equipment and, perhaps more importantly, that indirect attempts may still provide adequate and necessary Information. Results and descriptions of methods of more basic studies have been referred to elsewhere (Ch. 2).

The first part of this chapter thus provides a list of useful approaches to the problem of validating ring counting as an ageing method, as well as a brief discussion of the limitations inherent in each. The second part is a summary of experiences on how to select material, how to prepare otoliths for counting and how to perform the actual counting. Intentionally, methods applicable for routine analysis have been treated most thoroughly.

3.2 Validation of ring counting as ageing method.

Providing firm indications of a general correspondence between increment number and age is unfortunately not always straightforward. There are several approaches to the problem, most of which are mentioned below (Table 3.1).

Table 3.1. Approaches to the problem of validating increment counting as method for age determination.

I Relating increment number to real age.		
	a) Reared fish	- hatching time known
		- micro- or mesoscale rearing experiments
	b) Wild fish	- spawning period known (backcalculation of birthdates)
		- age known from seasonal marks in hard parts
		- age known from length frequency analysis.
II Relating increment number to natural or artificial marks.		
	a) Artificial marks	
		- marking by chemicals or special treatment of the fishes.
	b) Naturally occurring marks	
		- use of weekly, forthnightly, monthly or seasonal groupings.
III Comparison of growth curves.		

We would like to stress the importance of considering the different alternatives prior to doing validation studies. In the numerous cases where "the best" approaches are inapplicable, worthwhile studies can and should be conducted. Rather rough indications may be sufficient for many uses, and thus, one may find the more imprecise validation procedures acceptable.

Clearly, the most useful approaches are based on an independent knowledge of absolute age, from which a plot of increment number vs. age can be obtained. Now, the only way to know the exact age of individual fish is to know their hatching times. This is possible in controlled experiments only, with species for which successful rearing procedures have been or can be developed. A number of workers have applied this method (Brothers, Mugiya and Yamada, 1976; Taubert and Coble, 1977; Bakman, 1978; Tanaka, Mugiya and Yamada, 1981; Campana and Neilson, 1982; Neilson and Geen, 1982; Marshall and Parker, 1982; Geffen, 1982; Radtke and Dean, 1982). Added to the advantage of knowing the age exactly, one may be able to investigate responses to varying growth rates, behaviour patterns and environmental conditions.

The basic disadvantage of this approach is that the conditions offered to the fishes often differ markedly from those seen in nature. This may have substantial effects on growth, and survival (either positive or negative compared with rates seen in nature), and may also lead to rather drastic behavioural changes.

Attempts to mimic the natural habitat while still being able to monitor environmental parameters have been made in large basins, plastic bags or enclosed natural ponds (see Grice and Reeve (1982) for review). Obtaining material from such experiments may be an acceptable way of, at least partly, avoiding the negative effects of small-system rearing. Studies of otolith growth based on such material include those by Liew (1974), Gjøsæter (1981, 1982), Gjøsæter and Monstad (1982) Geffen (1982), Rosenberg and Haugen (1982) and Bergstad (1983).

One may, however, in some cases use wild-caught fish for which the approximate age is known. Age may be determined from seasonal marks or from knowledge of the spawning period of the population. A prerequisite for using the former method is that seasonal marks exist, which normally limits its usefulness to temperate and boreal fishes normally not younger than a year. Pannella (1971), Brothers, Mathews and Lasker (1976), Blacker (1975), LeGuen (1976), Timola (1977) and Gjøsæter (1979, 1982) applied this method, counting primary increments between annuli. For tropical species seasonal marks may either be too difficult to differentiate from randomly occurring structures or be missing entirely, making knowledge of spawning period the only means of determining real age (Steffensen, 1981; Penney and Anderson, 1981; Brothers, Mathews and Lasker 1976; Dayaratne, 1983).

The disadvantage of these approaches is that the precision of the age-estimates is usually rather low. Tropical species often show protracted spawning periods, and the age-variation within a sample taken after the main season may accordingly contain fish of widely varying age. The plot of increment number vs. time since max. spawning will normally show a wide scatter of points, and, in the most severe cases, make the basis of conclusions rather weak. In exceptional cases, however, local populations with a short spawning season occur, thus reducing the age range within samples to acceptable levels. The spawning activity should be monitored rather closely to obtain information on maximal age variation to be expected.

Length frequency analysis or modal progression analysis is useful for determining age in a number of species from boreal and temperate regions, usually in stocks showing rather short spawning periods. One may in these cases relate primary increment number to age estimated from body length. (a method often applied to verify the presence of seasonal marks (Bagenal, 1978)). In the cases where length frequency analysis has proven inadequate, as in many tropical species, this approach will be virtually useless. Even in the cases to which this approach apply, one would never expect to obtain more than rough indications of average rate of increment deposition.

It should therefore, be clear that age estimates from independent sources are often either difficult to obtain or of little use due to low precision. If possible, other approaches to the problem should therefore be considered.

One potentially very useful approach involves marking the otoliths of a group of fishes. At intervals of days or months following the marking, one may recapture fish, count the number of increments added since the marking and relate this to the number of days of liberty. A plot of increment number vs. time would give indications of average deposition rate since release, independent of knowledge of real age.

Otoliths may be marked by chemical agents, of which perhaps the most useful is tetracycline. Offered in the feed, injected, or added to the tank water this will leave permanent traces visible as a fluorescent band under ultraviolet light. Wild and Foreman (1980), Campana and Neilson (1982) and Campana (1983), successfully applied this method in studies of two species of tunas (Katsuwonus pelamis and Thunnus albacares) and starry flounder (Platichthys stellatus) and rainbow trout (Salmo gairdneri) respectively, while Struhsaker and Uchiyama (1976) were apparently less successful with the nehu (Stolephorus purpureus). It appears that choosing the right concentration of tetracycline is rather critical.

An even simpler method of marking otoliths was utilized by Victor (1982). Apparently, depriving fish of light, food or temperature cycles as well as supplemental feeding left distinctive structures on the otoliths. This may be a very useful approach, provided the treatment does not cause permanent harm to the fishes.

In some cases regular natural structures occur. Pannella (1971, 1974) and many others (see Table 2.1) accordingly grouped the smallest recognizable increments into approximately weekly, bimonthly, monthly and annual units. This may, at least in some species, give useful indications of what should be regarded as daily structures. The problem, however, is to determine with certainty which checks can be related to which time markers. If composite units are found consistently, as for instance forthnightly bands (Rosenberg, 1982), one should perhaps consider using these for ageing rather than the more diffuse daily increments.

A completely different approach which may be acceptable, although rarely very accurate, involves comparing growth data obtained by otolith ageing with ones obtained by other means. If the growth curves are similar, one would assume that age was on the average correctly determined from the otoliths (see Brothers, Mathews and Lasker, 1976; Ralston, 1976; Penney and Anderson, 1981; Gjøsæter, 1981a and b; Uchiyama and Struhsaker, 1981 for examples). Now, one problem is that growth information obtained by independent means either does not exist, or is at most rather approximate. The method should, therefore, be used with caution, at least in the cases where existing data point to large variations in growth.

To conclude; all the approaches mentioned above have definite limitations. Thus, their appropriateness will vary with the species or life stage in question, as well as with the resources available. Still, it appears rather obvious that knowledge of age (or the time period since marking) is the best basis for validation studies. Thus, we recommend that some effort should be made to obtain such data before choosing other approaches. As stated earlier, this is not to say that efforts along other lines are of no use. Taking care to avoid potential pitfalls, highly relevant information may be obtained by these means.

3.3 Sampling procedures.

The sampling program should primarily aim at facilitating a proper testing of the specific hypotheses of the study in question. Obviously, attempts to validate the ageing method will require very different approaches than a routine study of age distribution, mortality patterns or growth of a stock. In the latter cases, it may be essential to consider most thoroughly aspects like temporal and spatial distribution, gear selection and allocation of a usually very limited cruise time to cover the appropriate area and

time period. A further discussion of these points is, however, beyond the scope of this report. Some useful references include Bagenal (1978) and Cochran (1963). We will, however, discuss a few general points relevant to validation studies. It should be realized that the sampling scheme will have to be modified following the choice of method to approach the problem (as outlined in the previous section).

Prior to selecting material for validation studies one should preferably have determined the optimal width of the age interval to be covered, the distribution of samples within this interval and the sizes of the individual samples. Although this may seldom be possible in the strictest sense, even rather crude structuring of the sampling is likely to improve the quality of the results.

Clearly, to avoid undue extrapolation, the age interval should be as wide as possible, at least covering ages relevant to future age-distribution or growth studies. This is important since results obtained for one life-stage may not be valid for another (Pannella, 1971; Brothers, 1979; Gjøsæter, 1982; Bergstad, 1983; Campana, 1983). Periods of profound morphological or physiological change (metamorphosis, maturation, spawning, slow-growth seasons etc.) should be included to check whether any changes in increment formation happen.

The degree to which a strict control of the distribution of samples within the interval is possible will vary. In mark-recapture experiments one is often forced to rely on recaptures from commercial catches, thus one has neither control of the age-distribution nor the size of the samples ("single fish samples" may be frequent). In other cases, like in studies of experimental populations or single cohorts, one may be able to sample at predetermined intervals. Nevertheless, sampling should be particularly intense in periods of growth change, as mentioned above, since interpolation may be unsafe in such cases. Thus, the time intervals between samples should not necessarily be equal at all stages.

In studies covering a rather wide age range in which only two samples, one at the beginning and one at the end, are taken, information can be lost and results may be misinterpreted.

Ideally, sample size should be determined from previous knowledge of the variation in ring number estimates at each particular age. Such exact information is rarely available, and no formal treatment is thus possible. Still, one should take care to increase sample size when within-sample variation increases, whatever the reason may be (counting error, real variation between fish of same age, variable age, sampling artifacts etc.). Relatively large samples permit safe estimation of mean values and variances, and thus increase the amount of information considerably.

These are all ideal aims of a sampling program and may serve as general guidelines. One is, however, often faced with situations where a direct control of the sampling is impossible (i.e. pre-collected material, sampling program breaking down), and hence one has to accept whatever is there. One may certainly still obtain useful information. The general principles mentioned should be considered, though, in order to determine to what extent sampling inadequacies affected the results.

3.4 Preservation of fish or otoliths

Having collected a sample of fish, appropriate procedures should be followed to avoid loss or reduction of quality of the otoliths. The main objective is to select a process which makes the otoliths remain intact by preventing decalcification or reduced distinctness of the growth increments.

Working with relatively large specimens it may be convenient to extract the otoliths at the sampling site. Until preparation for counting they can be stored either dry in paper or plastic envelopes or in a suitable liquid (alcohol has been widely used). The alternative for smaller specimen (including fish larvae) is to preserve the entire fish, either by freezing, drying or a liquid preservative.

Drying (oven-drying at 60°C or so for several hours) may be acceptable for fish larvae when the proper facilities are there and if no further measuring of morphological characters is needed. The larvae can be dried and stored on glass slides. To facilitate otolith extraction they should be softened by a drop of distilled water.

Freezing is a good alternative if freezer capacity allows and accidental thawing can be prevented (for example during transport). It may, therefore, be most suitable for work in the laboratory or in rather permanent field facilities. As with drying, pronounced morphological changes occur during freezing and thawing. These should be weighed against the need for morphological data.

The advantage of drying and freezing is that the danger of decalcification is minimal. Some common liquid preservatives may, however, become acidic, and cause severe surface erosion or total loss of the otoliths. Thus, aquous solutions of formaldehyde cannot be recommended (even when buffered). Ethanol, usually made slightly alcalic by addition of small amounts of an inorganic base, is widely used and well suited although its properties as a fixative are inferior to those of formaldehyde. Concentrations in the range 70-95% have been used in previous studies; we would recommend rather concentrated solutions since the fish' interstitial water will gradually lower the concentrations. Also, the body cavity of large fish should be punctured to allow effective fixation. During longterm storage the preservative should be changed at intervals.

As several liquid preservatives, ethanol causes body skrinkage; thus, conversion tables between fresh and preserved lengths (or other dimensions) should be worked out (see for example Worthman, 1980). This is especially important for larval fish studies, where, in addition to shrinkage, severe body deformation may occur. (As number of recent accounts of these problems have appeared; notably Theilacker (1980) and Hay (1982)).

3.5 Extraction of otoliths.

A number of methods for extracting otoliths have been described. General accounts are provided by Bagenal and Tesch (Bagenal, 1978) among others. Considering the wide variations in skull morphology among fishes, there will always be need for modifications of described methods. Working with fish larvae, the absence of skin pigment facilitates localization of the otoliths prior to dissection. They appear as small shiny bodies somewhat lateral to the brain, and can usually be extracted fairly easily with fine needles, working under a dissecting microscope, (see for example, Geffen, 1982; Laroche, Richardsen and Rosenberg, 1982).

3.6 Choice of otoliths for study.

Fishes have three pairs of otoliths; the sagittae, the lapilli and the asterisci (saccular, tricular and lagenar otoliths respectively) (see Pannella (1980) for review and useful references on otolith morphology). Sizes and shapes vary widely among species and among life-stages of a species. Sagitta and lapillus have proven the most useful for ageing purposes, while asteriscus is either very small and fragile or shows irregular incremental patterns (neither is it present at hatching in all species (Bergstad, 1983)).

While sagitta is usually larger than lapillus in adults (exceptions are found in the Ostariophysi, according to Pannella (1980)), the opposite may be true in larvae of some species (Bergstad, 1983). Therefore, the otoliths should, at least initially, be identified according to their position in the labyrinth (see for example Pannella, 1980) rather than to size differences.

Sagitta may be the most useful otolith, at least in adults, due to its size. Lapillus is, however, the preferred in some species (Brothers, 1979; Brothers and McFarlane, 1981; Brothers and Victor, 1982).

There may be differences between the two as regards both number and distinctness of the growth increments, and they may not be equally suitable for treatments like grinding (see next section). Differences in increment numbers could stem from unequal time of first

ring formation or unequal deposition rates (or both). In larval cod, Gadus morhua, L., the latter appears true (Bergstad, 1983); both otoliths get their first increment around the time of hatching but subsequently show unequal and variable deposition rates. In other cases, like with two coral reef fishes (Thalassom bifasciatum and Halichoeres bivittatus) and the pescada, Plagioscion monti, no differences in numbers were found (Victor, 1982; Worthman, 1980).

There is reason, however, to recommend selecting one kind of otolith for routine use, since switching may introduce substantial systematic errors in some cases. If possible, comparisons should be made.

A fraction (usually 5-10%) of the otoliths will be crystalline, show abnormal shapes or have too diffuse rings to allow any reliable counting. These should be rejected. Note, however, that the other otolith of the pair can be quite acceptable. Hence it may be wise to extract both otoliths from all fishes to prevent excessive loss of information. Systematic differences in ring number between the left and right otolith of the same kind have never been reported (Campana and Neilson, 1982; Neilson and Geen, 1982; Geffen, 1982; Bergstad, 1983).

3.7 Preparation of otoliths prior to counting.

The choice of technique for preparing otoliths for counting primary increments depends on the size and shape of the otolith, and on how the otoliths will be read.

Three methods have been in general use:

1. Light microscope view of an optical or real section of the otolith.
2. Light microscope view of an acetate replica of a polished and etched otolith section.
3. Scanning electron microscope view of a polished and etched section.

The last method is very time consuming and requires expensive equipment. It is therefore not very suitable for routine use and will only be mentioned briefly here.

The first method is usually to be preferred, although when the otoliths are thick and robust, the second is quicker and may provide as good results as those obtained by other methods.

Initially, the otoliths should be cleaned of all adhering tissues. This can be done by fine needles working under the dissecting microscope. The otolith should be immersed in water or ethanol.

Otoliths below a certain size are usually sufficiently transparent to allow direct viewing by optical sectioning. This holds for larval otoliths and for some flat juvenile otoliths (in cod larvae, for instance the upper size limit was around 0.3 mm (diameter)). In such cases, the otoliths should be mounted under coverslips in a clear mounting medium (immersion oil, Pro-texx, EPON, or others) on clean glass slides. (It may be advantageous to make the concave side point upwards to improve quality). The choice of mounting medium may not be critical, although certain kinds, notably Canadabalsam, have proven less suitable than others (Canadabalsam may be or become acidic). Use of permanent, hard media, like Pro-Texx or EPON, has the advantage of reducing the risk of fracturing (on the other hand they may be difficult to obtain from retailers). When mounting in immersion oil, the coverslips should be secured properly to avoid sliding and possible breakage (wax or nail-varnish have been used successfully). Before mounting, proper drying; preferably for half an hour or more at 60°C, is absolutely essential.

Larger otoliths from juveniles and adults are normally too thick to allow counting of the innermost increments after being prepared as above. Grinding, etching and sawing have been used with success to reduce the thickness in a controlled manner, or to obtain true sections in a predetermined plane. Since the morphology and size of the otoliths varies,

no single method can be rated as generally superior to others. Some trials will have to be done when starting to work on a new species, and there is clearly room for much imagination when adapting the general procedures outlined here.

After mounting the dried otolith in a hard medium (e.g. microkitt, EPON) grinding can be done by using wet-dry carborundum paper (grit 400-800), metallurgical lapping film, aluminum oxide, carborundum powder etc. The carborundum paper is immersed in water and grinding can be done by hand or by using a mechanical device specially adapted for the purpose (e.g. the one described by Neilson and Geen, 1981). Metallurgical lapping film is used dry both for grinding and polishing. Aluminum oxide and carborundum powder is moistened and the grinding is done on a slightly uneven surface like a sintered glass plate. An alternative grinding method involves using a dentist's drill equipped with fine-grit finishing discs. This is a very quick method, but seems most suitable for rather large and robust otoliths.

Wild and Foreman (1980) used 5N hydrochloric acid (HCl) for etching the otolith to expose increments in its thickest parts. The acid was applied for short periods of time with a brush, taking care not to dissolve the increments along the edge. This may be a useful and simple method, but clearly requires great caution to avoid undue decalcification.

Sawing is applicable to rather large otoliths only. The otoliths must be firmly embedded in hard epoxy resin, polyester or a suitable kind of glue. Major disadvantages include the danger of fracturing the otoliths, the problem of positioning the otolith to obtain the exact section needed and the fact that a high-speed electrical saw is required.

Grinding may still be the simplest and most straightforward method, at least for juveniles and adults of species having rather small or thin otoliths (e.g. most clupeoids, mackerels and flatfishes). Trials should be made to determine whether a true section has to be made by grinding from two opposing directions or if grinding one surface is sufficient. Grinding two sides can be done by going through the following steps: mounting otolith concave side down - grind convex side - dissolve mounting medium - remove otolith and embedd again ground surface against the slide - grind concave side - dry, and apply mounting medium and coverslip. Sometimes the otolith can be fixed on a strip of two sided tape while grinding the concave side. Thus it needs to be mounted only once, making the overall procedure less elaborate.

Following grinding, polishing may be required. Commercial metal polish is suitable as well as diamond paste. Note, however, that using fine-grit carborundum paper or metallurgical lapping film may in many cases make polishing superfluous.

Grinding and polishing should be done at the dissecting microscope, since frequent viewing is required to avoid wearing out marginal increments or grinding deeper than intended. The grinding should just expose increments in the core region.

After grinding, the distinctness of the increments can sometimes be improved by etching the ground surface with dilute HCl (1-5%) or solutions of EDTA. Tests should be made to determine whether etching is at all necessary. When using the acetate replica technique, etching is always required. It has also often proven beneficial for Scanning Electron Microscope (SEM) studies.

The acetate replica technique is probably most suitable for very robust adult otoliths for which grinding can be very time consuming. The method was utilized by Pannella (1971) and has proven very useful in many situations. The otolith has to be broken or sawed to get a section through the nucleus. This section should cut the growth surfaces at right angles, and there should not be any discontinuity in the increments. The simplest solution will usually be obtained by breaking the sagitta at the center along a dorsoventral direction (XZ plane in Fig. 3.1), using thumb and index finger of both hands or by pressing a needle downward in the central area.

If this section does not give satisfactory results one should try to break the otolith in a sagittal direction (the yz-plane in Fig. 3.1) or to grind it down to the xy-plane.

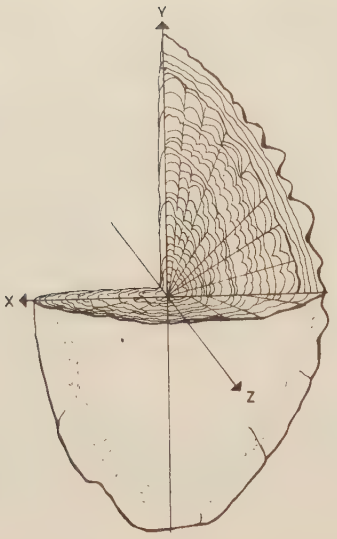


Fig. 3.1. Section of an otolith showing axes referred to in the text.

If the otolith is strongly curved, this grinding may be easier if the otolith is first broken dorsoventrally and the two parts are treated separately.

When a proper surface is obtained the following procedure should be followed:

Grind, and if necessary polish, the surface till it gets smooth and flat. Then etch it by 1% hydrochloric acid for 20-80 sec. Repeated trials may be necessary to find the best etching time. EDTA could also be used for etching. After etching, clean the otolith with water and dry it.

Place a piece of acetate paper on a glass slide and soak it with acetone. When the paper is soft, press the etched surface of the otolith firmly into the acetate paper. After drying (about 30 min) the otolith may be removed carefully, and the print protected by a coverslip sealed by transparent tape.

Otoliths intended for studies by the scanning electron microscope could be sectioned and etched as described above. For further preparation (mounting and counting) the manual of the SEM equipment available should be consulted.

3.8 Counting growth increments.

To beginners (and at intervals to most otolith students) the search for regular patterns in otoliths may be a frustrating experience. Except in a few beautiful cases, one is faced with a great variety of structures; some very distinct, others rather diffuse. Consulting the literature may be equally frustrating, since the general lack of consistency of definitions and terms prevails. Pannella (1980) discusses much of interest at this stage. The simplest (and, some would say the most vague -) definition of what we accept as a primary increment is: "The smallest concentric unit composed of a narrow dark band and a wide light band". This is thus far consistent with descriptions found in the litterature (Pannella, 1971; Barkman, 1978; Wilson and Larkin, 1980; Schmidt and Fabrizio, 1980; Penney and Anderson, 1981; Gjøsæter and Tilseth, 1982; Laroche, Richardson and Rosenberg, 1982; Geffen, 1982 and others), and may represent what is seen by scanning electron microscopy (SEM) as 'discontinuous' and 'incremental' zones (Tanaka, Mugiya and Magaba, 1981; Radtke and Dean, 1982). The width of primary increments varies within the range .25 to 25 µm (Brothers, 1979); tropical fishes normally showing wider increments than temperate or boreal fishes.

These structures are, however, not necessarily always formed daily, although most increments which are determined to indeed represent daily growth, fit this definition. Now, problems arise when more diffuse structures occur within these primary increments (cases often described as 'daily' increments containing 'subdaily' units), and when several kinds of increments occur on the same otolith (see for example our description of Sardinella spp.).

The flow-chart in figure 3.2 should illustrate a simplified sequence of questions to be answered in a validation study. As outlined, it is clearly most relevant to cases in which increment number can be related to real age (approximate or exact) or to a known time period (mark-recapture studies). Not all boxes need to be considered in all studies; in fact in the more "easy" cases only those belonging to the left panel may be needed.

Anyway, initial inspection of the otoliths is needed to define which structures to count, and to get an indication of whether or not the selected structures are at all likely to represent 'daily' marks. A search for weekly, monthly or other bands should be included at this stage. Following initial tests comes an analysis of the total number of otoliths available ('Main run'). We believe that this general approach will be the most rational in most cases, since an immediate treatment of the entire material, may lead to excessive waste of time and effort.

We recommend that several person take part in the initial tests. This is to determine whether different eyes, either immediately or after discussions, recognize the same incremental patterns. Accordingly, the different counters should at first neither communicate during counting nor know the 'desired' result (ring number corresponding to a certain age or time interval). If immediate agreement is reached, what we call a 'pre-run' should be made. If this check is positive, the next step should be the 'main run'.

Before proceeding any further, it may be appropriate to discuss a few aspects concerning the use of the microscope. One should put some effort into finding the optimal illumination, level of magnification and focussing technique. The goal is, of course, to be able to recognize and separate increments in as large parts of the otolith as possible.

Except when the otoliths are labelled with tetracycline, white light is used overall. Using a polarizing filter may be beneficial in many cases (our experience indicate that this at least greatly improves quality of photographic prints). The level of magnification will vary, but should not be changed drastically during routine counting since this may induce considerable bias. Also, one should note that increasing magnification leads to greater focussing problems and optical effects which in many cases can be misinterpreted as ring structures.

The focussing problems can be considerable, especially when entire otoliths or rather thick sections are used. No general recommendations can be given here, since the optimal procedure will vary from one species to another. One will normally have to focus slightly while counting along the radius of an otolith. This is acceptable as long as one is confident that each increment is included only once.

Although the microscope is the essential piece of equipment, the efficiency of data collection and storage can be improved further by using video systems and/or computerized recording. (see for instance Methot and Kramer, 1979; Lough et al., 1982). We stress, however, that these systems are by no means necessary to conduct otolith studies, certainly not for validation studies. A very useful, and reasonably inexpensive, attachment for discussing otoliths is a 'discussion tube', which allows two persons to examine the same otolith at the same time. An alternative is discussing photographic prints of the otoliths.

So much for the more technical matters. Now, problems may already have arisen in the initial stages. Either the choice of structures is difficult to make, and there is no immediate agreement between counts, or the 'pre-run' reveals significant deviations from a 'daily' pattern. In the former event, problems may be transitory if the otoliths are

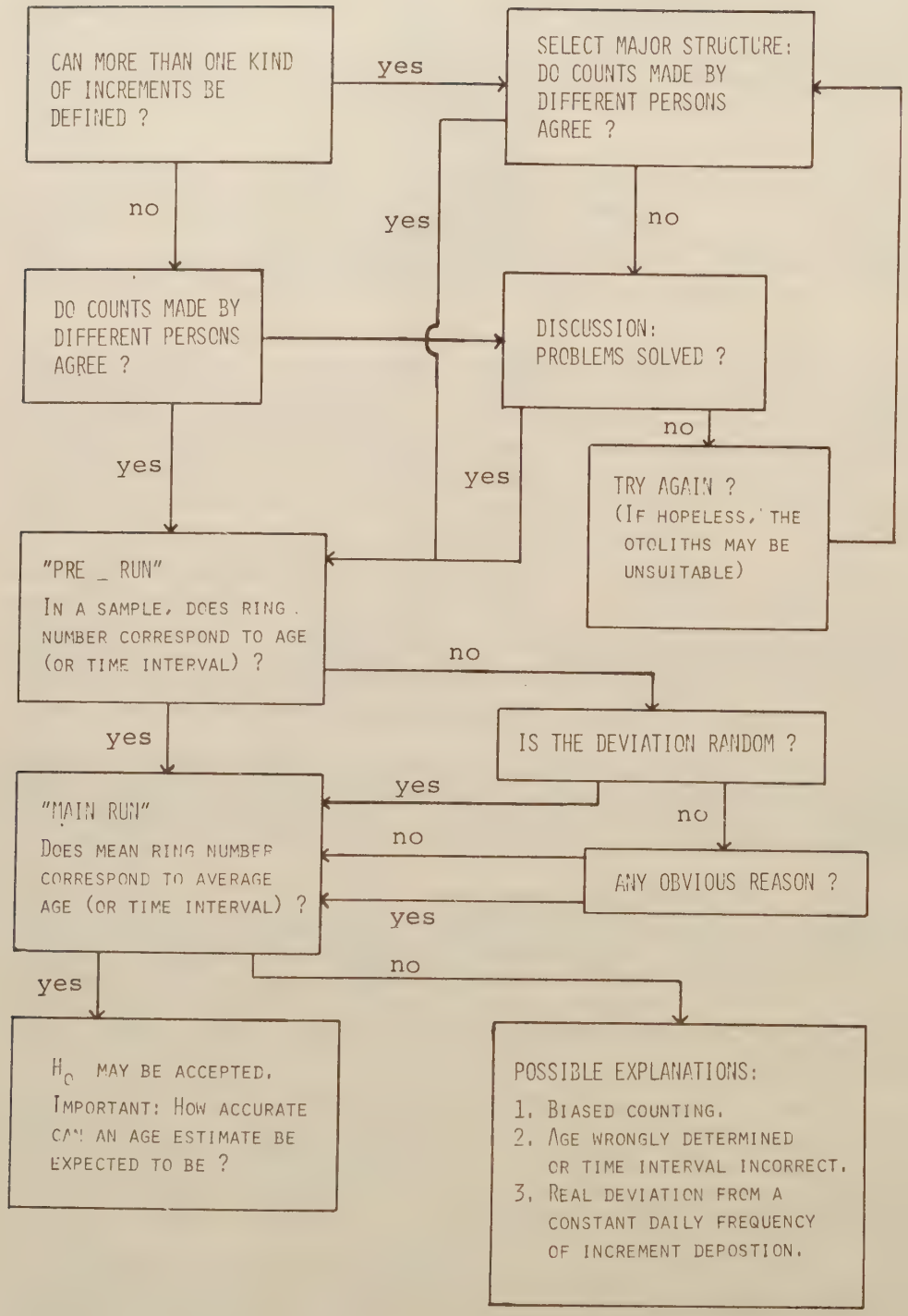


Fig. 3.2. Generalized illustration of considerations involved in validating increment counting on otoliths as ageing method.
H_0: Otolith increment number equals no. of days of age, or no. of days passed a fixed point in the fish'life.

iscussed, and an agreement on which structures are to be accepted is reached. The most ronounced structures occurring consistently should be chosen as a first alternative at his stage (note, width in the range .25 - 20 µm). In some cases, however, confusion revails even after repeated trails (agreement within approximately 5% is still not eached). In the most hopeless (and fortunately rather rare) cases one may have to egard the method as useless already at this stage; that is, the conclusion may be that ageing, if at all possible, would be hopelessly imprecise.

Consistency between counts may be reasonable, however, while deviation from what would correspond to a 'daily' pattern seems high. The deviation may be random; that is, there is a large standard deviation to the mean number of increments, yet the mean still approximates age (or time interval). This should not cause too much concern, since the estimate is still unbiased, only less precise. If, however, pronounced bias towards either too low or too high numbers is indicated, one should check whether there is any immediately obvious explanation.

Too low counts might result from systematic inability to register increments in parts of the otolith, like near the focus and close to the edge. There may also be differences in ring numbers between sectors on the otoliths; i.e. starting from the focus, counting towards, the dorsal edge may give lower numbers than counting towards the anterior or posterior tips. Too high increment counts may result when including, intentionally or accidentally, rather faint structures. The opposite is true when real 'daily' marks show low or variable degree of distinctness. Working with fish larvae, bias towards too low or high numbers may result when the first increment is formed a few days after or before hatching respectively (see for example Brothers, Mathews and Lasker,1976, Radtke and Dean, 1982).

One should, if none of these alternatives apply, not exclude the possibility that real deviations from daily deposition rates exist, either in the entire period or in parts of it.

Following the 'pre-run' one may, then, decide to start all over again searching for structures which fit the model better. This is probably feasible when another structure occurs consistently, but was neglected in the earlier stages of the process. In other cases (possibly most cases) one should continue to perform the 'main run' without worrying about random error or bias.

The 'main run' includes counting increments on all otoliths selected for study in a fashion which minimizes effects of all known sources of error. Especially important, as mentioned previously, is taking care that the 'expected' or 'desired' result is unknown to the persons involved. Thus, the counters should be informed of which increments to accept and how to count them (which sector of the otolith, which magnification and so on), but nothing more, such as the age of the fish or its length. No such data should be printed on the slides nor on worksheets used by the counters. Also, one should take care not to present the slides in a sequence which indirectly reveals information of age or size, but rather randomize them prior to performing the counting. A quick and safe alternative involves marking and presenting the slides according to a list of code numbers obtained from a series of random digits (see almost any statistics text, for example Sokal and Rohlf, 1969 (Statistical tables)).

Now, the counts should be repeated at least once, preferably more. Involving several persons also at this stage may not always be necessary, but will normally add strength to the basis of further conclusions. In cases where the 'pre-run' reveals negligible counting error, repeating counts more than once as well as involving other counters, may be regarded as unnecessary. These are, however, exceptional cases. The choice usually stands between two ways of utilizing available effort, either by increasing the number of counts for a single person or increasing the number of persons (counting one or more times each). If differences are small between one person counts while being relatively larger between different persons counts, one should increase the number of counters rather than the number of countings per person. In other cases the opposite may be

appropriate. Experience from work with larval and juvenile cod indicates that differences within and between persons add equally to the total observed counting error. In such cases, involving several persons may in principle appear unnecessary. It does, however, increase the credibility of the results, since it provides a useful check of whether or not different persons can recognize the same structures. Thus, we would normally recommend involving two or more counters, rather than letting the same person count several times.

3.9 Interpretation of results.

The data from the 'main run'; usually averages of independent ring counts, should now be related to age (or no. of days of liberty). A plot of increment number vs. age may form the basis for further interpretations. In general, information on the average rate of increment deposition can be drawn from such a plot by inspecting the slope of the line fitted to the data. Assuming on the average constant daily ring deposition, one would expect a straight line with a slope equal to unity. If this is found immediately, one may accept the hypothesis of figure 3.1. for the case in question.

Attention should now be directed at assessing how reliable and precise a future age estimate can be expected to be. Some relevant information can be drawn from an analysis of the scatter of points in the 'age vs. no. of incr.' plot. Essential here is appreciation of the factors responsible for the scatter; these are outlined in Table 3.2.

In most instances only counting error is open for direct estimation. (Although maximum variation in age may be known). It is essential to note, however, that such information per se says nothing about the accuracy with which age of single fish is determined, only of how precisely the ring number is determined. The accuracy (or reliability) depends on how stable the relationship between increment number and age (or time) really is. That is, on the extent of individual variation in ring numbers within and among groups of fish of equal age. Unfortunately very few approaches to the validation problem allow such variation to be estimated; normally additional experiments are needed (the exceptions are rather rare situations when both counting error and age variation is low). One should take care, however, not immediately to interpret all the variation seen in increment number vs. age plots as resulting from counting error.

Table 3.2. Sources of error of the mean number of increments at a given age (including cases when only approximate age is known).

	Source of error	Comment
(1)	Real variation in age within a sample.	Problem in all studies in which age is only imprecisely determined.
(2)	Counting error or bias.	Causes: Incorrect choice of structures, systematic or unsystematic inability to recognize or register all patterns really present etc.
(3)	Unequal age at first ring deposition between individuals	Important in larval and early juvenile studies only. Usually a minor source of error.
(4)	Real differences in increment number between individuals of same age. (between or within groups)	Causes: Either (3) or differences in ring deposition rate in parts or entire period prior to collection. (includes interruptions)

Now, the results of the main run may indicate that ring numbers as determined sometimes or always provide biased estimates of age or time period. In a plot as outlined above, either the linearity assumption is violated (i.e. no straight line can be drawn between points), or the slope of the line is significantly different from unity (as determined by the appropriate regression analysis, see for example Sokal and Rohlf, 1969). A less severe case of bias is seen when the slope is equal to one, but the elevation of the line makes all age estimates seem either too high or too low. As outlined previously, this is usually a consequence of counting bias (a constant number of increments is either lost or added) or of the fact that increment deposition is initiated before or after hatching. Also, interruptions of increment deposition may have occurred prior to the sampling period. (Note that these problems are not necessarily detected when using mark-recapture techniques, only when absolute age is used as reference). What makes these cases less troublesome than others is that the errors can easily be compensated for by adding a constant factor.

If age or time interval is rather exactly known, the deviations causing a slope different from one or a curvilinear relationship may be conclusively determined as real. Thus, deposition rate either varies or is constant at a level lower or higher than 1 ring$\cdot d^{-1}$. Artifacts can, however, produce similar 'unnormal' relationships, especially when the independent reference data are imprecise or biased. Take for instance cases where successive samples from what is assumed to be the same cohort are used. Sampling artifacts such as area or gear selection may lead to: a) as the fishes grow, only the larger and older fish of the cohort are caught, b) same as a) but only the smaller and younger are caught (the larger escapes gear) c) combination of a) and b). Situation such as a) and b) would give straight lines having slopes higher or lower than one, respectively, while c) might give curvilinear relationships (or indeed, a wide unsystematic scatter). Apart from this, the basis for assuming that all samples come from the same cohort may be weaker than expected, which in turn may lead to very disturbing results. These examples illustrate that what appears superficially to be very serious deviations from an average daily pattern of otolith growth, may not be reason to immediately outrule the method as such. It emphasises, however, the need for proper knowledge of the limitations of the validation methods and the need for careful analysis of the results.

4. CASE STUDIES

4.1 Sardinellas.

a. Description of otoliths

The otoliths of all four Sardinella species studied, (S. sirm, S. albella, S. gibbosa and S. longiceps) had similar shape and structure. They are anteroposteriorly elongated with two rostra (usually one longer than the other) on the anterior end and a rounded posterior end. The central part of the otolith is thickest, with a concave proximal surface and a convex distal surface. On the distal side there is a depression in the central area, making it thinner and more transparent than the surrounding part of the otolith.

The size of the otolith in a medium length specimen of some Sardinella species is given below.

Table 4.1. Size of some Sardinella otoliths.

Species	Total fish length (MM)	Greatest length of otolith (MM)
S. sirm	150	2.5
S. albella	110	2.0
S. gibbosa	110	1.7
S. longiceps	110	2.8

The otolith microstructures of these species also follow the same pattern. The nucleus is situated somewhat anteriorly, just behind the two rostra.

The nucleus appears as a black spherical spot, usually with a 6-8 µm diameter. Around this nucleus the otolith growth increments are deposited concentrically.

The nuclear region has an outer radius of 60-100 µm and is rather transparent. The 14-24 rings in this region are uniformly narrow, about 4-6 µm.

In Sardinella albella some faint, very narrow rings appear in between these clear rings. However, the narrow rings were not counted as they disappear with the change of the focus. The same phenomenon was noted for S. longiceps.

In S. gibbosa the rings in the nuclear region are about 9-10 µm wide and appear thicker than in the other two species.

Between the nuclear region (A in Fig. 4.1) of the otolith and the following region (B) there is an abrupt change in the appearance of the rings. In this second region the rings are more opaque and typically 16-20 µm thick although they do not maintain a uniform thickness all around the otolith.

Within some of the thicker rings found in region B there occassionally appear about 3-4 very narrow rings, which are assumed to be subdaily. The thick rings are originally widely spaced but both ring thickness and intervals are gradually reduced. The transition to the outer region (C in Fig. 4.1) with more narrow rings is therefore gradual. Towards the outer edge of the otoliths of larger fish, the rings become very narrow, transparent and very densely packed. Sometimes irregular interruption of these narrow rings could be observed.

In large otoliths of S. albella the peripheral narrow rings sometimes seem to follow weekly or fortnightly patterns but are not considered units for calculation as they do not have a sufficiently high regularity.

b. Methods of preparation

The sagittae were dissected out by folding the operculae upwards and making a deep incision along the ventral side of the cranium. The otoliths were cleaned, dried and stored in paper envelopes.

The otoliths were then fixed to a glass slide, convex side upwards, using a drop of transparent mounting medium. In fixed otoliths of S. sirm, rings could be seen even without grinding. The otoliths of all species except S. longiceps were ground on 400-600 grit carborundum (wet/dry) paper placed in water, to increase the clearness and visibility of the rings. In S. sirm, only a little grinding was required to make the rings clear and readable. In S. albella and S. gibbosa the otoliths were ground carefully under a dissecting microscope, until the thicker bands in the central area were clearly visible. The ground otoliths were cleaned in 70% alcohol, dried for about 10 minutes and mounted in Canadabalsam. However, the visibility and clearness of the rings in these mounted otoliths decreased after about one month. This could be due to the acidity of the medium, or because the otoliths were not properly dried before mounting. In later preparations the ground otoliths were dried in an oven (50°C) for about 30 minutes before mounting.

In S. longiceps extracted otoliths were put in plastic envelopes and stored dry until examination. These otolith were found to be very fragile, and difficult to grind without tearing off the rostra. However, after dehydration for about 24 h. in absolute alcohol and mounting in Canadabalsam, it was possible to read even the biggest of these otoliths (from fish of 12 cm) without grinding.

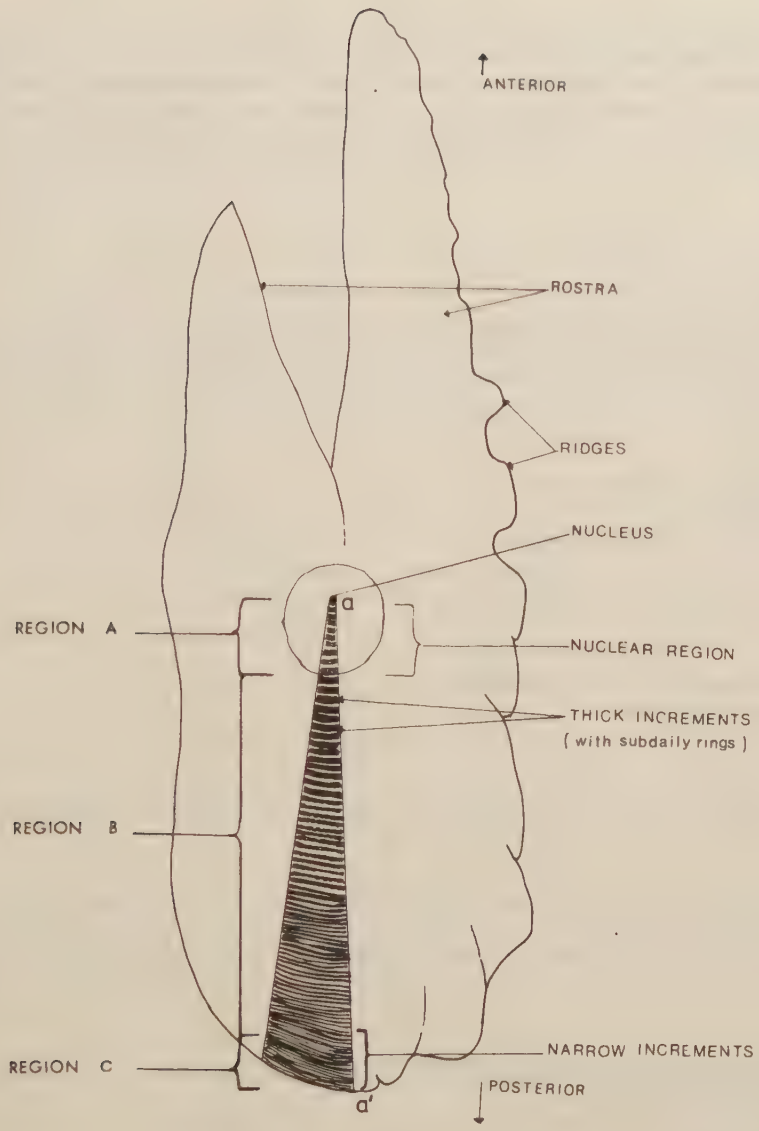


Fig. 4.1. Schematic drawing of a Sardinella otolith (sagitta). Rings are shown along the section on which the counts were made. (a-a) - axis along which the radii were measured.

c. Interpretation

The primary growth rings in Sardinella species can be clearly seen after preparation. One dark and one light band together is considered as one ring.

The otoliths of Sardinella species are usually best counted at a lower magnification (x 250). However, in larger otoliths where the rings close to the edge are very narrow it is necessary to use higher magnification.

Rings are usually counted from the nucleus to the posterior edge of the otolith. In good preparations rings can usually be counted along the same radius throughout the otoliths but where this was not feasible, counts could be conducted along several radii by following clear rings from one section to the other.

The narrow rings which may appear within the thicker bands in the central region (Region B on Fig. 4.1) are interpreted as sub-daily rings, and therefore not counted. This interpretation gives the most reasonable estimate of growth and spawning season and also the best correspondance between the ring count increase and sampling interval (See Verification under 4.1.3).

4.1.1 Sardinella sirm

a. Consistency of counting

Otoliths of S. sirm are easy to read and there is a high consistency between the ring counts made by two persons. Counts were made by two readers on the same otoliths and the following results were obtained.

Table 4.2. Sardinella sirm. Consistency of ring counts made by two readers.

	Reader 1	Reader 2
Mean ring count	295.63	290.5
S.D.	35.82	28.5
95% confidence limits	±15.89	±12.66
Total number of otoliths	22	22

The replicability of two counts made at different times by the same reader was also tested similarly.

The two counts were not significantly different (paired observation t-test, $p<0.05$).

The two counts of two samples by the same reader were also not significantly different (Table 4.3).

Table 4.3. Sardinella sirm. Consistency of ring counts made by the same reader.

	First reading	Second reading
Sample A		
Mean ring count	166.69	163.69
S.D.	27.98	29.09
95% confidence limits	±16.93	±19.16
Total number of otoliths	13	13
Sample B		
Mean ring count	297.17	299.75
S.D.	47.10	36.6
95% confidence limits	±20.37	±15.85
Total number of otoliths	23	23

b. Verification

When the same population is sampled several times the time difference between the sampling dates should correspond to the estimated age difference, and therefore give a linear relationship with a slope of 1.

Regression analysis was used to test the relationship between the time of sampling and the estimated age of the fish, and a linear relationship with a coefficient of determination of 0.821 was obtained (Fig. 4.2):

$$y = 1.094\ x + \text{const.} \qquad N = 132$$

y = estimated age in months
x = sampling time

The standard deviation of the slope was 0.047, and the slope is therefore not significantly different from 1. The sample taken in July 1981 belongs to a different cohort and is not included in the analysis. The correlation between time lapse and calculated age difference strongly indicates that the rings observed are formed daily. Further evidence for this is also found in reasonable growth estimates and estimates of spawning season given by this method of ageing (P. Dayaratne, in prep.).

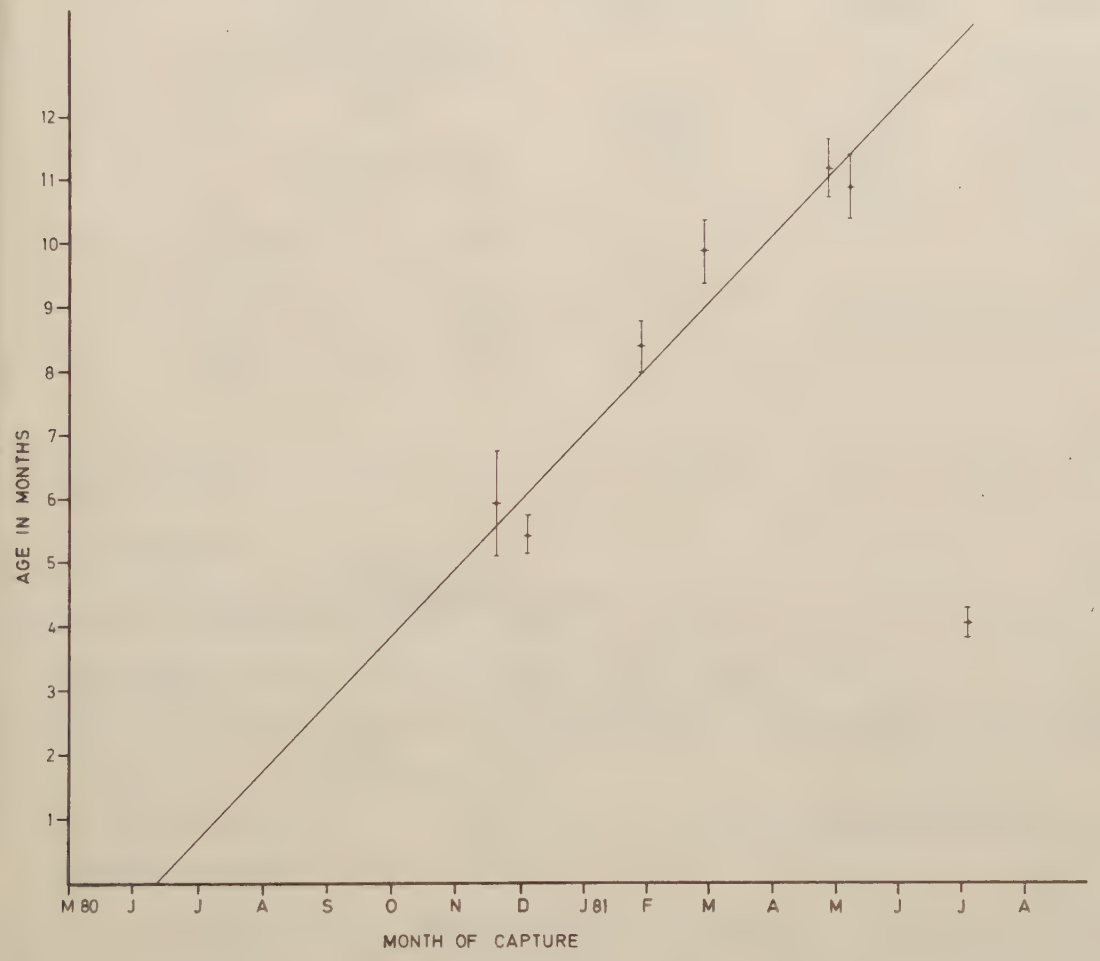


Fig. 4.2. Relationship between age by otolith dating and time of capture of Sardinella sirm from Negombo.

c. Correlation between otolith radius (r) and fish length (l)

The radius of the otolith of S. sirm was measured from the nucleus to the posterior edge using an ocular micrometer. In our study using fish 13.7-22 cm long a linear relationship was found between the fish length (l cm) and otolith radius (r mm):

$$l = 11.5r + 0.35$$

The coefficient of determination was 0.914.

d. Growth according to the ageing

Growth of Sardinella sirm was estimated by using the length/age data, back calculation and also from length frequency distribution, (ELEFAN program, Pauly and David, 1981). Von Bertalanffy's growth curves $l_t = L_\infty(1-e^{-K(t-t_0)})$ were separately fitted to the data and the growth parameters K, L_∞ and t_0 were obtained graphically (Beverton & Holt 1957).

The values obtained are given in Table 4.

Table 4.4. Sardinella sirm. Growth parameters of the von Bertalanffy's equation.

Growth parameter	Length/age data	Back calculation	Length/frequency distribution ELEFAN program
K (year)	2.5	3.74	3.5
L_∞ (cm)	22.0	22.0	23.5
t_o (year)	-0.062	-0.068	-0.217

No other reliable estimates of the growth rate of S. sirm seem to be available.

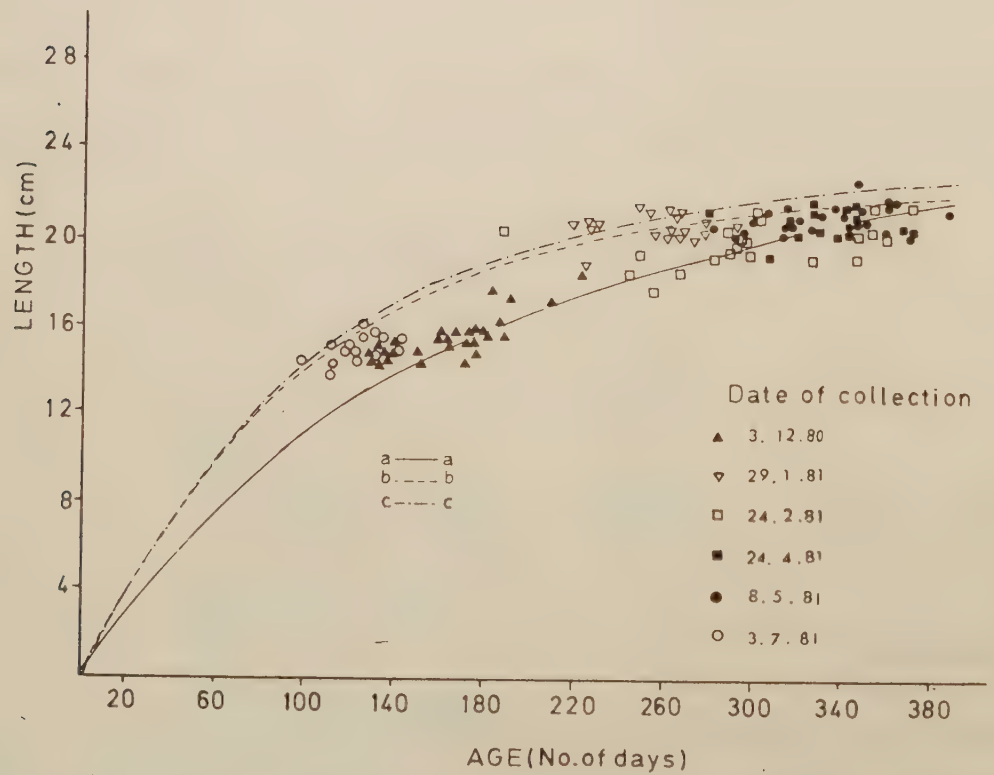


Fig. 4.3. S. sirm. Plot of estimated age versus fish length (TL) growth curves shown were obtained by using
(a-a) - Length/age data
(b-b) - Back calculated data
(c-c) - Length/frequency analysis

4.1.2 Sardinella albella

a. Consistancy of counting

Otoliths of S. albella were not as easy to read as those of S. sirm, but still the countings usually showed a high replicability.

Otoliths from two samples were counted by two readers, and the results are given in Table 4.5.

Table 4.5. Sardinella albella. Consistency of ring counts made by two readers.

	First reading	Second reading
Sample A		
Mean no. of ring	132.36	132.14
S.D.	17.98	11.13
95% confidence limits	±10.38	± 6.43
Total Number of Otoliths	14	14
Sample B		
Mean no. of ring	361.0	361.3
S.D.	25.09	29.02
95% confidence limits	±12.49	±14.44
Total Number of Otoliths	18	18

The two countings were not significantly different (paired t-test, t=0.049 (A) and t=0.052 (B)).

b. Verification-

See under S. gibbosa (4.1.3)

c. Correlation between otolith radius and fish length

A close linear relationship was found between the otolith radius (mm) and the fish length (cm) for fish 6-12.9 cm long.

The relationship, 1 = 10.7 r - 0.38 had a coefficient of determination of 0.949.

d. Growth versus age

Growth of S. albella was estimated using the length/age data and from back calculation (Table 4.6). Von Bertalanffy's growth curves were fitted using graphical methods (Beverton & Holt, 1957).

Table 4.6. Sardinella albella. Growth parameters of the von Bertalanffy's equation.

Growth parameter	Length/age data	Back calculated data
K (year)	2.16	2.03
L_∞ (cm)	12.4	13.8
t_0 (year)	0.03	-0.01

No other reliable information seem to exist on the growth of this species.

4.1.3 Sardinella gibbosa

a. Consistency of the counts

The otoliths of S. gibbosa were usually easy to read although not as easy as S. sirm.

Three samples of otoliths were counted by two readers and the results are given in Table 4.7.

Table 4.7. Sardinella gibbosa. Consistency of ring counts made by two readers.

	Reader 1	Reader 2
Sample A		
Mean age	202.4	192.07
S.D.	23.35	24.4
95% confidence level	±13.48	±14.09
Total Number of Otoliths	14	14
Sample B		
Mean age	205.4	207.7
S.D.	20.28	18.17
95% confidence level	± 8.37	± 8.27
Total Number of Otoliths	20	20
Sample C		
Mean age	67.24	76.26
S.D.	7.84	13.63
95% confidence level	± 3.24	± 6.57
Total Number of Otoliths	19	19

The two counts for samples A and B were not significantly different (paired observation t-test, $p<0.05$) whereas a difference was found for the third set of otoliths (paired observation t-test, $0.02<p<0.05$).

b. Correlation between otolith radius and fish length

A close linear relationship was found between fish length (1 cm) and the otolith radius (r mm) in fish 5.3 - 13.2 cm long:

$$1 = 14.99 \quad r - 2.29$$

The coefficient of determination was 0.960.

c. Growth versus age

The growth of Sardinella gibbosa was estimated by using the length/age data and from back calculation.

As with the other two species, Von Bertalanffy's growth curves were fitted to these data separately and the following growth parameters were obtained:

Table 4.8. Sardinella gibbosa. Growth parameters from the von Bertalanffy's equation.

	Length/age data	Back calculation
L_∞ (cm)	13.3	14.1
K (year)	3.90	3.49
t_0 (year)	0.016	-0.016

No other reliable information seem to exist on the growth of this species.

d. Verification, S. albella and S. gibbosa

As for S. sirm a verification of the age from primary growth rings was obtained by correlating sample interval with ring increase.

The relationships obtained for both S. albella and S. gibbosa were almost linear with a coefficient of determination $r^2 = 0.62$ for S. albella and $r^2 = 0.61$ for S. gibbosa.

The equations obtained were:

S. albella $y = 0.566\ x + \text{const.}$ N = 101

S. gibbosa $y = 0.697\ x + \text{const.}$ N = 86

Where y = estimated age in days, x = sampling time

Samples collected on 20.3.81 of S. albella, and 20.2.81 of S. gibbosa were not included in the above analysis as they seem to belong to different cohorts.

The slopes obtained were both significantly different from 1, indicating that the time difference between the samples does not correspond to the difference of estimated age. However, this difference in results could be explained by the sampling gear selectivity. The gear used in the Sardinella fishery was a small mesh gillnet with a stretched mesh size of 28 mm. Therefore, at the first sampling dates when most of the fish were near the lower end of the size ranges there was probably a strong selection towards the largest and consequently the oldest fish. If this is the case the slope of the regression lines given above should be expected to be lower than 1, especially for S. albella and S. gibbosa whose size ranges were 6-12.9 and 5.2-13.2 cm respectively.

The present results do not ascertain that the primary rings are formed daily, but taking the probable gear selectivity into considerations, it does not falsify the claim either.

The growth rates found for S. albella and S. gibbosa seem reasonable, as do the spawning seasons estimated from this ageing method (P. Dayaratne in prep.).

These estimates too may be altered by size selective catchability, and a firm verification can therefore not be given at present.

The similarity between the otolith structure of these species to those of S. sirm makes it, however, plausible to use primary rings for estimating age of S. albella and S. gibbosa.

4.1.4 Sardinella longiceps

a. Consistency of counting.

A sample of otoliths was read twice, within a period of about two months by the same reader. The following results were obtained:

Tabell 4.9. Sardinella longiceps. Consistency of ring counts made by two readers.

	Reading 1	Reading 2
Mean	128.17	133.74
SD	17.12	16.31
95% Confidence limits	± 5.68	± 5.41
No of otoliths	35	35

The counts were not significantly different (t-test $p<0.05$).

b. Verification of ageing technique.

The material consists of only one collection of 48 fishes with readable otoliths. It is thus not possible to use the method described for the other Sardinella species, to compare the mean number of ring increments with sampling time intervals.

Two indirect methods for testing the age estimates have been used: 1) comparison of the spawning time calculated from the aged sample with the known spawning season, and 2) comparison of the growth calculated for the sample with published growth data.

Based on the assumption that the increments begin at hatching, a daily augmentation of the rings would mean that these fishes hatched during March and April 1979. Little is known about the spawning time of S. longiceps in this area.

Limited investigations have shown that both spring-spawning and autumn-spawning take place in the western Arabian Sea, thus the calculated spawning time based on daily growth increments is not unreasonable.

c. Growth.

Fish length versus number of increments is shown in Fig. 4.4. All the fishes fall within a narrow length interval, and no functional relationship can be established between fish length and otolith size. In the length interval 10-12 cm there seems to be an increase in number of increments with increasing length. If the increments are daily, the fishes have grown to 10-12 cm in about 4-5 months.

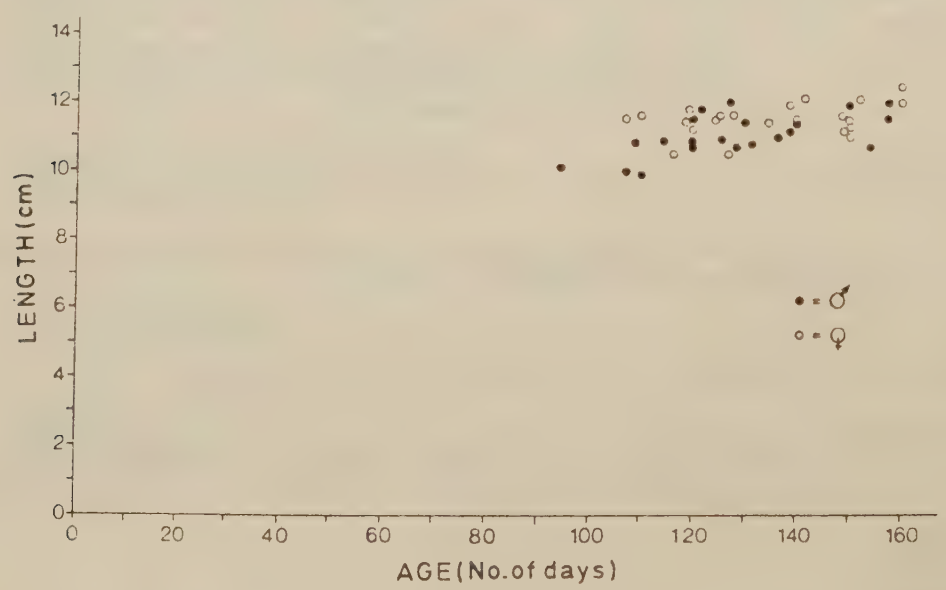


Fig. 4.4. Fish length vs. no. of increments on sagitta. Sardinella longiceps.

Assuming a linear relationship between fish length and otolith size, the otolith growth pattern shows that growth is slow the first month after hatching. The fishes then enter a fast-growing period which lasts about one month, whereafter the growth is slowly decreasing again. Based on a hatching length of about 5mm, the average growth until four month of age would be 0.8 - 1.0 mm/day.

Little is known about the growth of S. longiceps in the western Arabian sea. Reports on growth found in the literature are most often concerning Indian waters. In a number of such reports the most common method for growth study has been modal progression in length frequency histograms. The results of these studies show a large variation in estimated growth. Estimated maximum age varies from 2.5 to 14 years, length after the first year from 6.5 to 17 cm, and length at first spawning from 12-18 cm.

A direct comparison of growth between two areas is difficult when so little is known about differences in hydroclimate, food availability etc. Estimates of relative growth may, however, be made by this technique. S. sirm, which reaches about the same length as S. longiceps, seems to have a faster growth in Sri Lankan waters and has corresponding ring growth in the otoliths. It may be concluded that no reliable estimates of growth exist to verify or falsify the estimates from primary growth ring increments.

4.2 Decapterus russellii

a. Description of otoliths and interpretation of rings

The otoliths are thin and about 4-6 mm long in a medium sized fish. They have a prominent rostrum and a smaller antirostrum while the opposing end is rounded.

The primary growth rings are deposited concentrically around the nucleus. In the central part of the sagitta (Area A in Fig. 4.1) they were found in very regular groupings of 2-3 rings. This pattern comprises 40-80 first rings depending on the size of fish. Following this pattern alternating light and dark bands are visible, each of which is composed of 7, 10 or 14 primary rings.

In a fish of medium size these periodical bands are usually visible for the first 2/3 of otolith. The last third is comprised of very thin primary rings not grouped in bands. The otoliths were read under a microscope at a magnification of 640 x. All readings were done from the nucleus to the posterior rounded edge as the rings were more distinct in this section. Since the rings become thinner and more densely packed with increasing fish growth, it was found that the otolith readings from fish longer than 18.5 cm were very difficult and probably underestimate the true age.

b. Otolith extraction and preparation

Only the largest of the three otoliths on each side, the sagittae, was used for age estimates. These otoliths were removed by lateral dissection, rinsed, dried and stored in paper envelopes.

The otoliths were ground into thin sagittal sections either using a dentist drill equipped with polishing discs or using a whetstone. After grinding with the drill from the distal sagittal plane, the otoliths were fixed to a glass slide with a drop of transparent cement, exposing the proximal side which was in turn ground until the nucleus was reached. When a whetstone was used, the otoliths were moistened and ground from both sides before they were fixed to the glass slides. After grinding the otoliths were etched with a drop of 1% HCL, for 5-10 seconds. The acid removes most of the particles on the ground surface and the rings become clearer. They were then rinsed in water and mounted in DPX mounting medium.

c. Consistency of counting

Otoliths of young D. russellii were easy to read, whereas those of older fish were more difficult.

A few otoliths from older fish were read independently by two persons. The results were as follows:

	No. of rings		
Reader	Mean	SD	Range
1	506	55	409-650
2	525	74	421-563

The mean deviation between the readers was 20 rings (SD=61). Using a t-test for paired observations it was found that the results were not significantly different (t=0.97).

d. Verification and growth

Only the growth rates as estimated from otolith ring counting could be used to verify the age estimates.

Otoliths of 55 specimens in the length range 13.4 - 18.6 cm were studied to count primary growth rings (Fig. 4.5). If these rings are formed daily the age-length relationship shown in Fig. 9 is derived. Based on these data the following growth equation was fitted:

$$l_t = 24.8\ (1 - e^{-0.56\ (t + 0.10)})$$

This has good agreement with the growth curves fitted to length frequency data by Gjøsæter & Sousa (1983a), and growth rates as given by Tiews et al. (1970). This similarity in results provides some evidence that the counting of primary growth rings may give a reliable estimate of age.

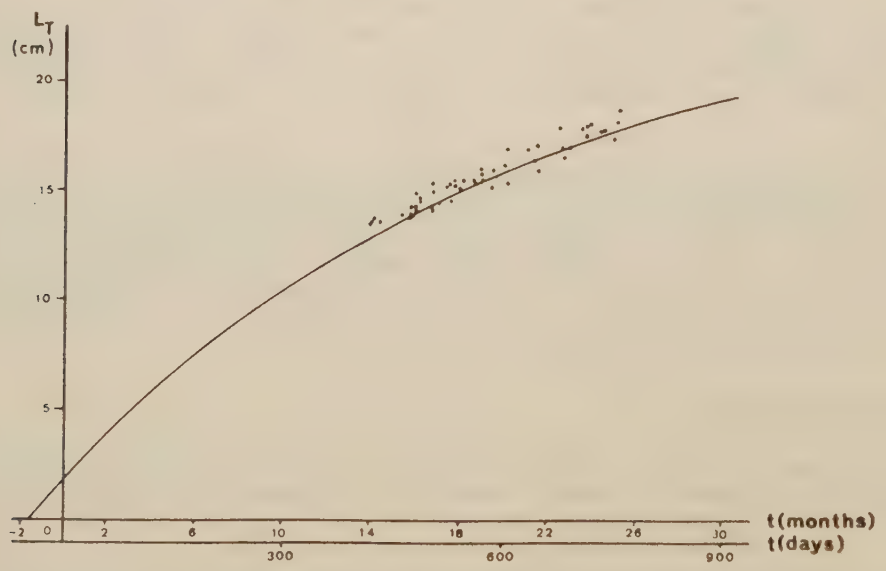


Fig. 4.5. Growth curve for D. russellii based on otolith readings.

4.3 Hilsa kelee

a. Description of otoliths and interpretation of rings

The otoliths are thin and about - mm long in a medium sized fish. They have a prominent rostrum and a smaller antirostrum while the opposing end is rounded.

The otoliths of H. kelee are difficult to read, because there are several types of rings, and it is not always clear which should be interpreted as primary rings. Usually there is

an inner zone (A in Fig.4.1) consisting of about 25-30 clear primary rings. After that a zone follows (B in Fig.4.1) where narrow rings are found and superimposed on them there are broader but more diffuse rings. In this area, counting the narrow rings will give about 3 times higher values than counting the broad rings. This zone than contain an average of 100 or 300 rings, depending of the interpretation. Outside this area the rest of the otolith contains narrow and densely packed rings (C in Fig.4.1).

Otolith extraction and preparation

The otoliths (Sagittae), were extracted by lateral dissection through the operculum and after being washed and dried they were stored in paper envelopes.

Two different techniques were used for the preparation of otoliths for readings. Some of them were ground on both sides using a wet whetstone. Grinding was alternated with observation under a microscope until the nucleus was reached. They were then glued to a glass slide with a drop of transparent cement and covered with DPX mounting medium. Others were first sticked to a glass slide with a drop of Microkitt and stiffened in an oven at 40°C for about 12 hours. They were then ground with the 600 Grit Carborundum wet/dry paper, alternating grinding with observation under a microscope. They were cleaned with alcohol and after being dried they were covered with Protexx mounting medium.

c. Verification and growth

Otoliths of specimens in the length range 8-19 cm were studied to count primary growth rings. The results for males, females and juveniles are given separately in Fig. 4.6.

As there apparently is no difference in growth between the two sexes, one single growth curve was fitted using graphical methods (Beverton and Holt 1957). The result was

$$l_t = 21.5\ (1 - e^{-1.1\ (t\ -\ 0.44)})$$

Otoliths from 64 H. kelee, with size 14-19 cm were also counted under the assumption that the broad bands of the intermediate zone are the daily rings.

The relationship between the two sets of counts is described using a linear regression with slope 1.02 ± 0.16 (5% conf. int.), and elevation 210 ± 8. The correlation coefficient is 0.858.

As there is an almost linear relation with a slope close to one between count based on the narrow rings in the intermeditate zone and those based on the broad rings, the K and L_∞ of the growth equation will remain nearly unchanged. The t_0 will change, and will in this case be close to zero.

It should be stressed, however, that recounting the otoliths from the young fish too, may change the parameters somewhat.

Growth estimates based on length frequency distributions give, K = 1.2 and L_∞ = 23 cm (Gjøsæter and Sousa, 1983b). This fits well with the results of ageing by means of otoliths. It therefore seems reasonable to believe that the rings in area C in Fig. 4.1 are formed daily. It is not verified which, if any, of the types of rings formed in area B should be regarded as daily. The very high t_0 obtained by counting the most narrow rings could, however, suggest that they are sub-daily.

4.4 Other species

4.4.1 Stolephorus sp.

A few specimens of the engraulid genus Stolephorus were sampled from the western Arabian Sea. The fork length range of the fish was 24-49 mm.

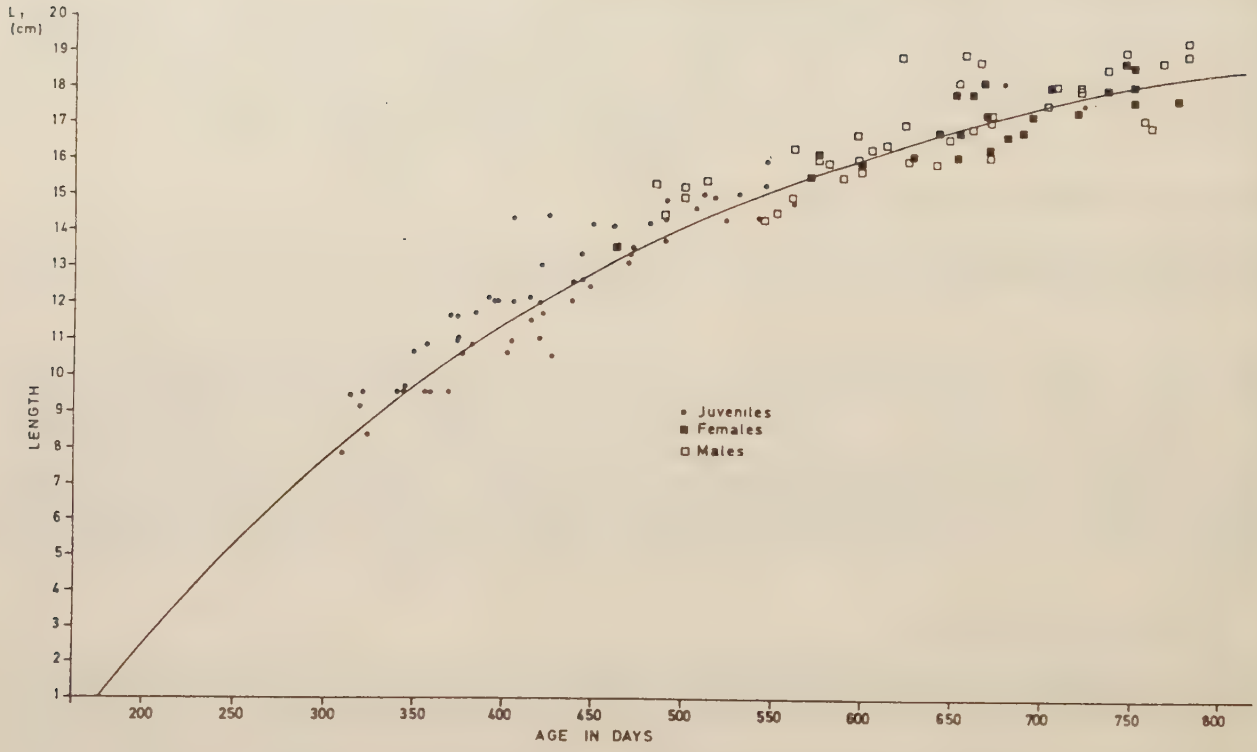


Fig. 4.6. Age-length distribution of H. kelee aged by means of otoliths. (Counting all visible rings).

Sagittae from specimens less than 30 mm long could be read without grinding and were mounted in Canadabalsam on microscope slides. The sagittae from larger fish had to be ground to count all the increments. This was difficult to achieve without losing one or both of the rostra of the otolith, due to the fragile structure.

a. Readability

These otoliths were difficult to read because they appeared to contain two kinds of zonation. Narrow increments, 2-4 mm thick could be seen, but there were also broader, more diffuse increments with thickness of 6-10 mm. From 1 to 5 of the narrow increments could be counted within the broader rings. To ensure that none of the two kinds of rings was an optical phenomenon, such as a lens aberration or light diffraction in otolith, acetate replicas were prepared from some otoliths. These replicas also showed rings of both types and therefore indicated that they were not artifacts.

Figure 4.7 shows the number of both types of increments plotted against fish length. No growth curve has been fitted to the data.

b. Comparison with other Stolephorus spp.

Struhsaker & Uchiyama (1976) found primary growth increments in S. purpureus in Hawaiian waters.

By counting the smallest increments from 2-4 mm wide, they verified a daily periodicity in a holding experiment. The thickness of these daily growth increments corresponds to the thinnest increments observed in Stolephorus sp. from the Arabian Sea. This, however, cannot be used as a confirmation of a daily periodicity in the thinnest increments, since the growth rate as well as the relationship between fish growth and otolith growth may be different for the two species.

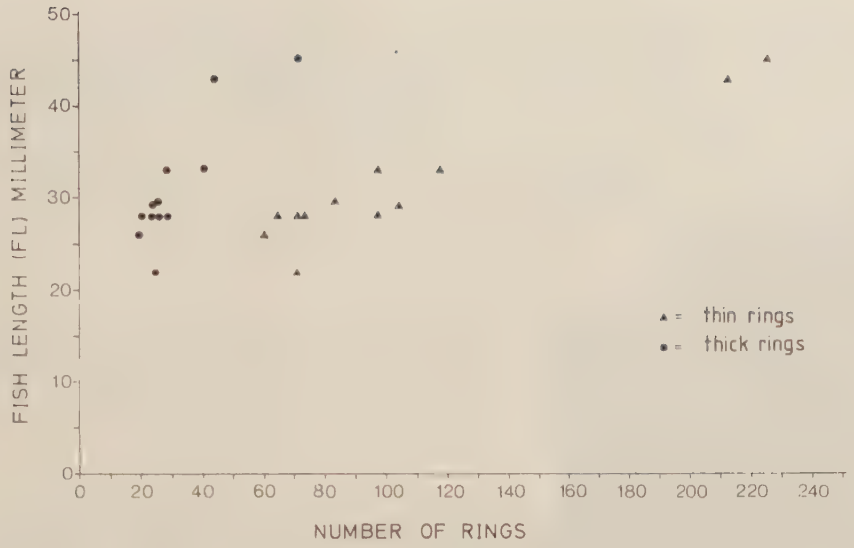


Fig. 4.7. Fish length vs. no. of increments for 12 specimens of Stolephorus sp.

c. Growth

The growth for three species of Stolephorus referred to in the literature has been compared in Fig. 4.8. The von Bertalanffy growth equations shown are from Jakarta Bay (Burhannuddin & Hutoms, 1975), Singapore Strait (Tham, 1966) and Palou (Muller, 1977).

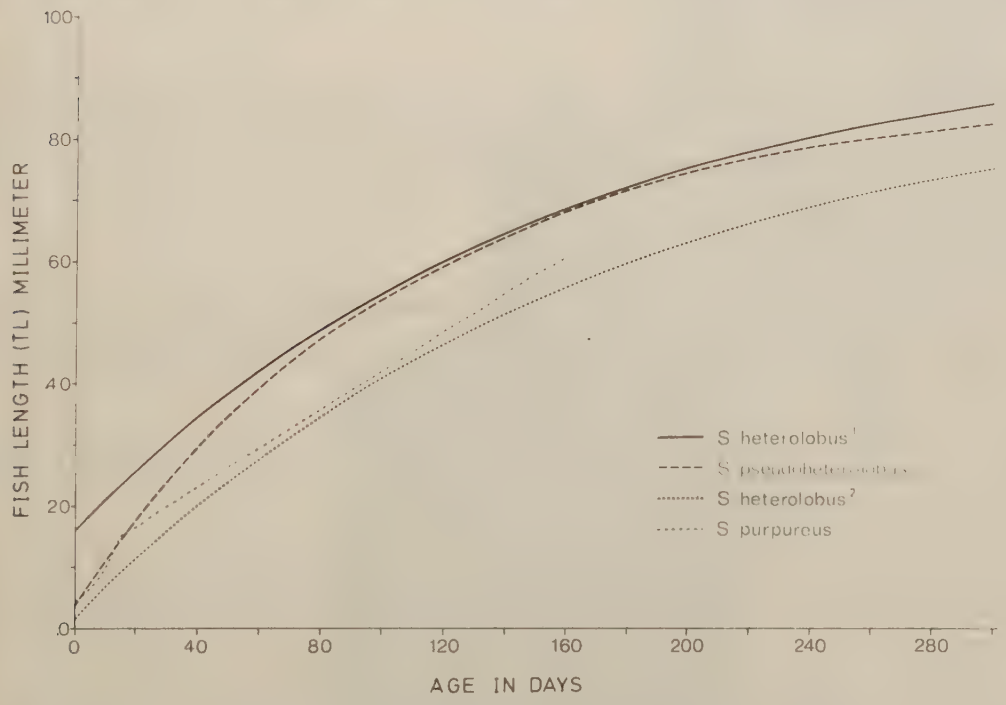


Fig. 4.8. von Bertalanffy growth curves for Stolephorus spp. based on reported investigations (see text for references) 1: Jakarta Bay, 2: Palau.

Comparing Fig. 4.7 with Fig. 4.8 reveals that if the thinner increments are daily they would predict a growth rate considerably slower than the estimated growth of the species referred to in the literature. If instead the thicker increments were daily, the growth would be similar to or a little faster than that of S. heterolobus and S. predoheterolobus from the Western Central Pacific and the eastern Indian Ocean.

As growth estimates of the genus Stolephorus from the western Indian Ocean are not available it is not possible to argue for a daily periodicity for either type of zonation. But, since similar increments are formed daily in the otolith of a related species in another tropical area, and it seems

possible to distinguish between the two types of increments, it is also likely that the otoliths could be used in ageing this species. However, it has yet to be determined, via one of the verification methods described in chapter 3 which of the increments types, if any, are deposited daily.

4.4.2 Nemipterus japonicus

The sagittae from a few specimens of Nemipterus japonicus were studied to investigate the feasibility of ageing by otolith growth increments. The fishes were sampled in the western Arabian Sea, and ranged in length from 10-16 cm.

The otoliths were rather large, the longest radius in the sagittae from specimens 10-16 cm being 2.5-3.2 mm, and difficult to grind properly. The best results were obtained using carborondum paper 400 and 800 grit immersed in water, and grinding both sides until the nuclear area was revealed. Two specimens were prepared for acetate replicas and scanning electron microscope studies.

Grinding of a sagittal section suitable for the light microscope reading was difficult due to the concavity of the sagittae. The grinding of a transverse or frontal section is, however, more time consuming.

An SEM picture of a transversal plane through the nucleus is shown in Fig. 4.8. From the nucleus to about half way to the perimeter very regular primary growth increments can be seen. In the outer region the increments are more diffuse, and several deep furrows may be noted in this area. These are more heavily etched than the surrounding material. The significance of these marks and their possible influence on ageing is uncertain.

Growth

Fish length is plotted against increments counts for 6 specimens (Fig. 4.10). Two growth equations for this species, referred to by Krishnamoorthi (1976) and Weber & Jothy (1977), are also shown. The highest growth estimates are for Indian waters while the other estimate describes growth near Malaysia.

If the primary growth increments are indeed laid down regularly, the counts indicate that males have a faster growth than females. This pattern is also corroborated by several growth studies of nemipterids.

Ben-Tuvia (1968) estimated the average length to be about 14-15 cm at the end of the first year and 18-20 cm after the second year, based on modes of length-frequency histograms from samples of this species in the Red Sea. These results are comparable to growth estimates from other areas and to growth calculated from the primary otolith increments.

With regard to the otolith preparation, it can be stated that the methods used are very time-consuming even for specimens of intermediate size. Sectioning may be better for the larger otoliths, but this has not been tested. Nonetheless, counting increments in otoliths more than 1-2 years old may in itself be unsatisfactory as a standard method of ageing.

Fig. 4.9. Scanning electromicrograph of a transverse section of a sagitta from N. japonicus. X195.
N- nucleus, E- edge.

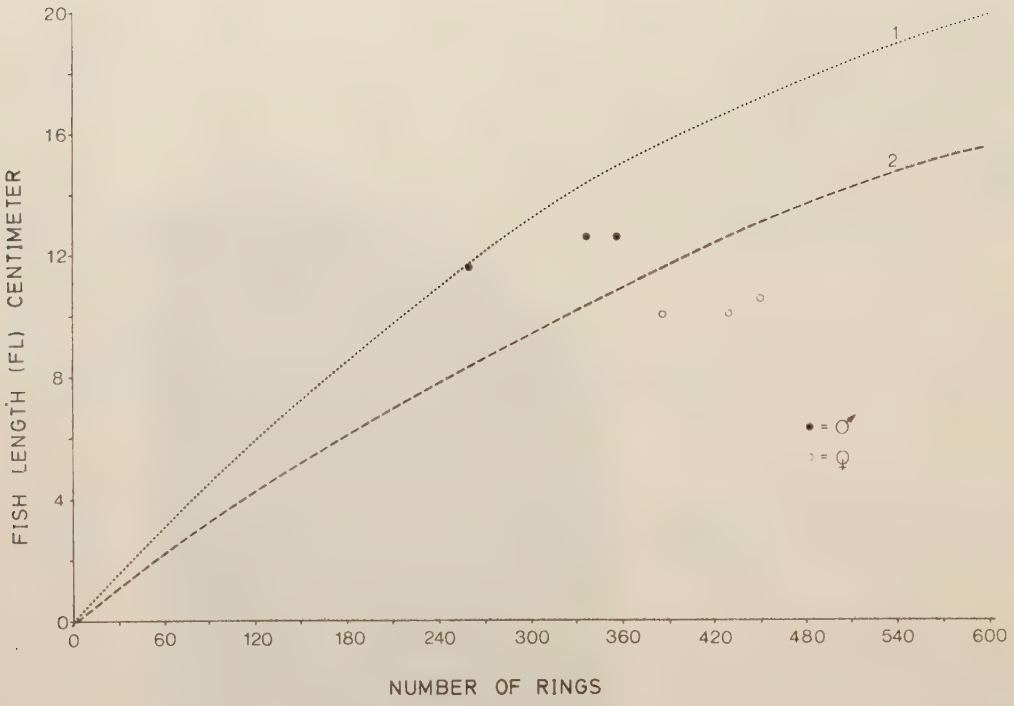


Fig. 4.10. Fish length vs. no. of increments for 6 specimens of N. japonicus. Growth curves reported by Krishnamoorthi (1976) (1) and Weber and Jothy (1977) (2) are shown.

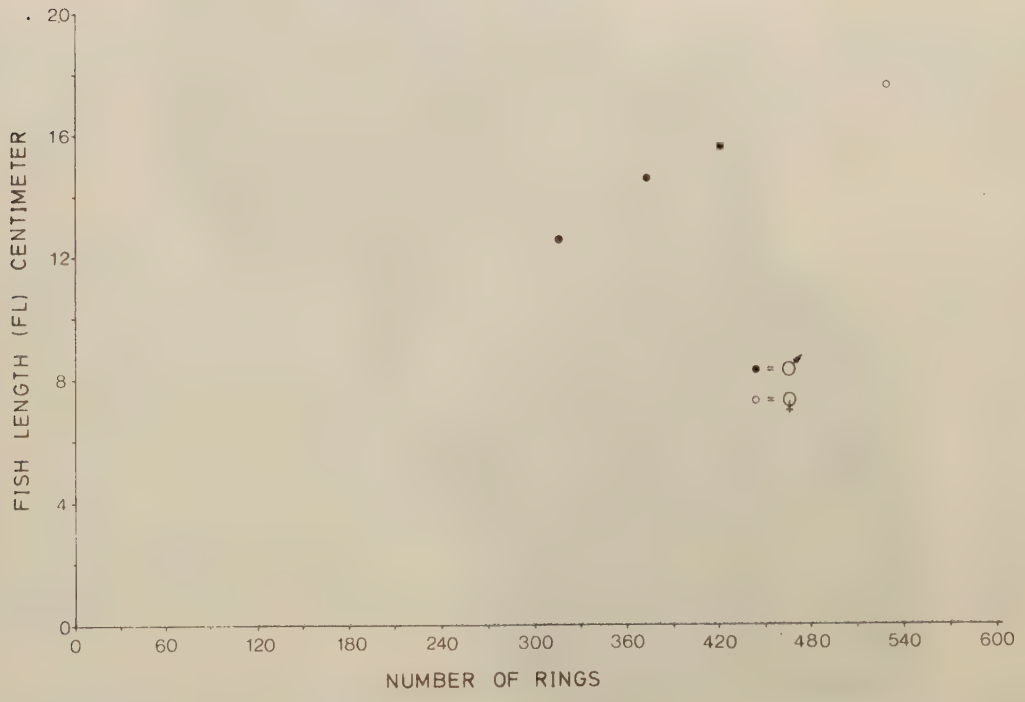


Fig. 4.11. Fish length vs. no. of increments for 4 specimens of Epinnula spp.

4.4.3 Epinnula sp.

The sagittal otoliths of five specimens of the Gemphylidae genus Epinnula sampled in the western Arabian Sea were extracted and prepared for inspection under the light microscope, using the same method described for N. japonicus. The fish were in the length range 13-18 cm. The sagittae were elongate, were flattened on both the distal and proximal sides, had one rostrum and had numerous small lobes along the edges. The size in relation to the fish length was comparable to the N. japonicus saggitae.

Four of the five otoliths could be read. The numbers of increments found are plotted against fish length in Fig. 4.11. The increments were relatively easy to detect, but the large number and the narrowness of the increments in the outer part of the otolith made total counts difficult.

No information on growth of these fishes has been available, and the hypothesis that the increments are daily cannot be tested. It may only be concluded that the otoliths of this deep water species contain primary growth increments which are possible to count by the described otolith preparation.

4.4.4 Tropical myctophids

The otoliths of 6 species of myctophids sampled in the Western Arabian Sea have been studied and their primary growth increments counted.

Some results are summarized here and details given in Table 4.10. For more information Gjøsæter (1981) should be consulted.

Table 4.10. Growth of some species of myctophidae.

Species	No. of Specimens	Readability	Mean length after 100 incr.	Mean length after 100 incr.	Von Bertalanffy's Parameters t_o days	K	L_∞ mm
Benthosema pterotum	108	Good	19^1 26^2	36^1 46^2	32^1 4^2	$.00573^1$ $.00389^2$	59^1 85^2
Benthosema fibulatum	50	Good	30	44	-3	.00628	61
Symbolophorus evermanni	16	Good	45	65	6	.00768	45
Myctophum spinosum	16	Good	-	70	-	-	-
Lampanyctus sp.	16	Fair	36	58	-	-	-
Diaphus diademophilus	4	Bad	-	-	-	-	-

1 = Gulf of Oman
2 = Gulf of Aden

Figs 4.12, 4.13, 4.14 and 4.15 show otoliths from some myctophid species.

General appearance of the sagittae

The sagittae from these six species are very similar. There is a nuclear area with a well defined transition to the outer perimeter of the otolith, marked by an area of very broad increments from 20 m in B. pterotum to 40 m in S. evermanni. The number of increments in the nuclear area was species-specific, and ranged from about 30 in B. pterotum to about 35 in S. evermanni. The increments in the nucleus may be difficult to see in larger otoliths unless the grinding plane goes accurately through the focus.

Fig. 4.12. Photomicrograph of an optical section of sagitta from a 7 mm larvae of Benthosema spp (X 320).

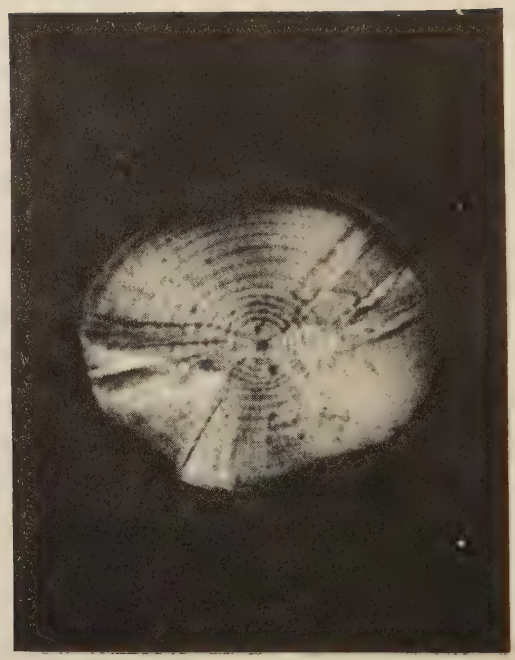
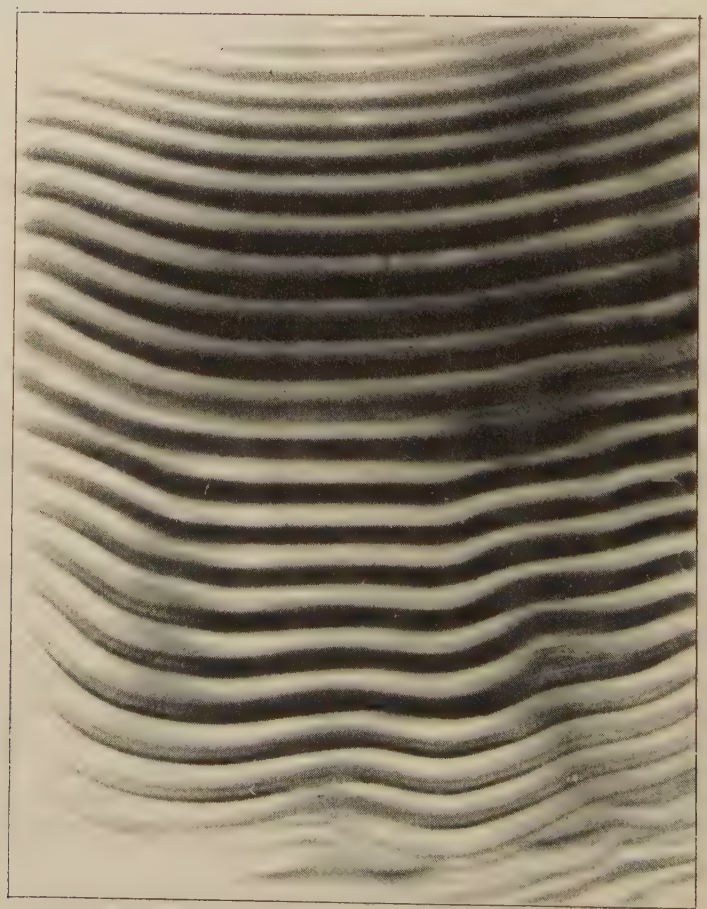


Fig. 4.14. Photomicrograph of a part of a sagittal section of sagitta from Symbolophorus evermanni (X320).



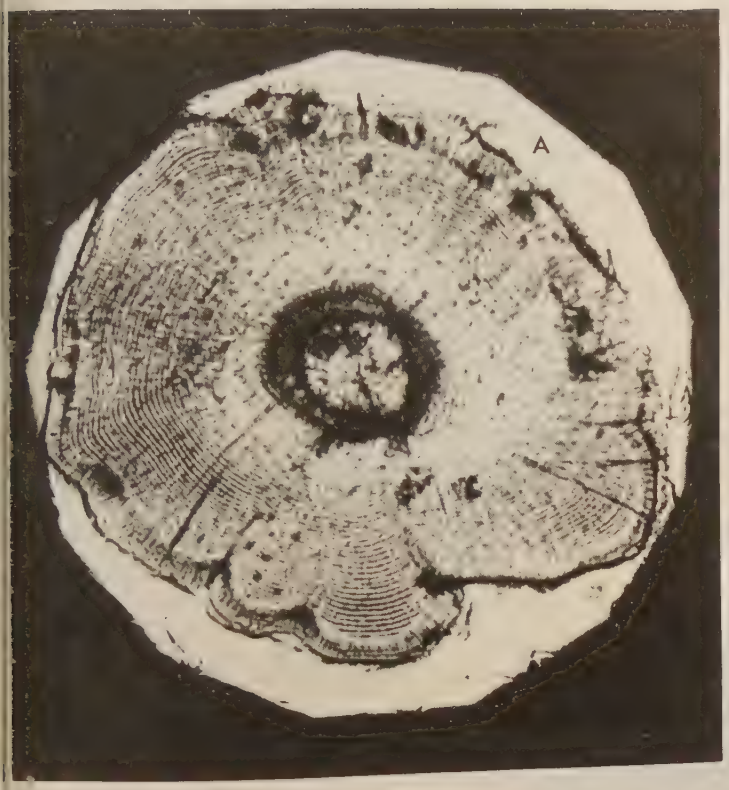
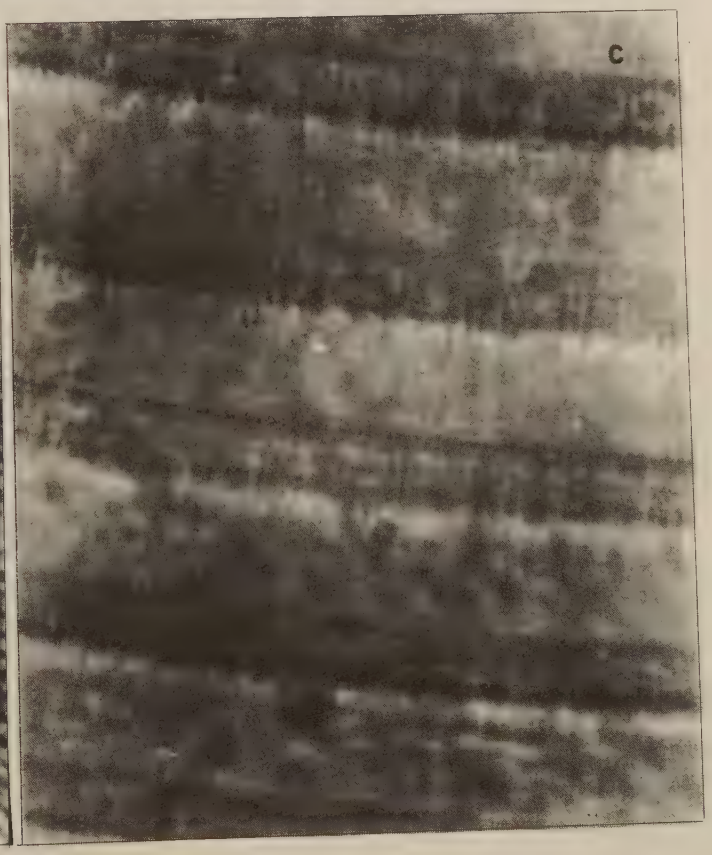


Fig. 4.13. Photomicrograph of sagittal section of sagitta from Benthosema fibulatum. A: total view X 64, B: part of A (antirostrum) X240, C: part of B X2240.



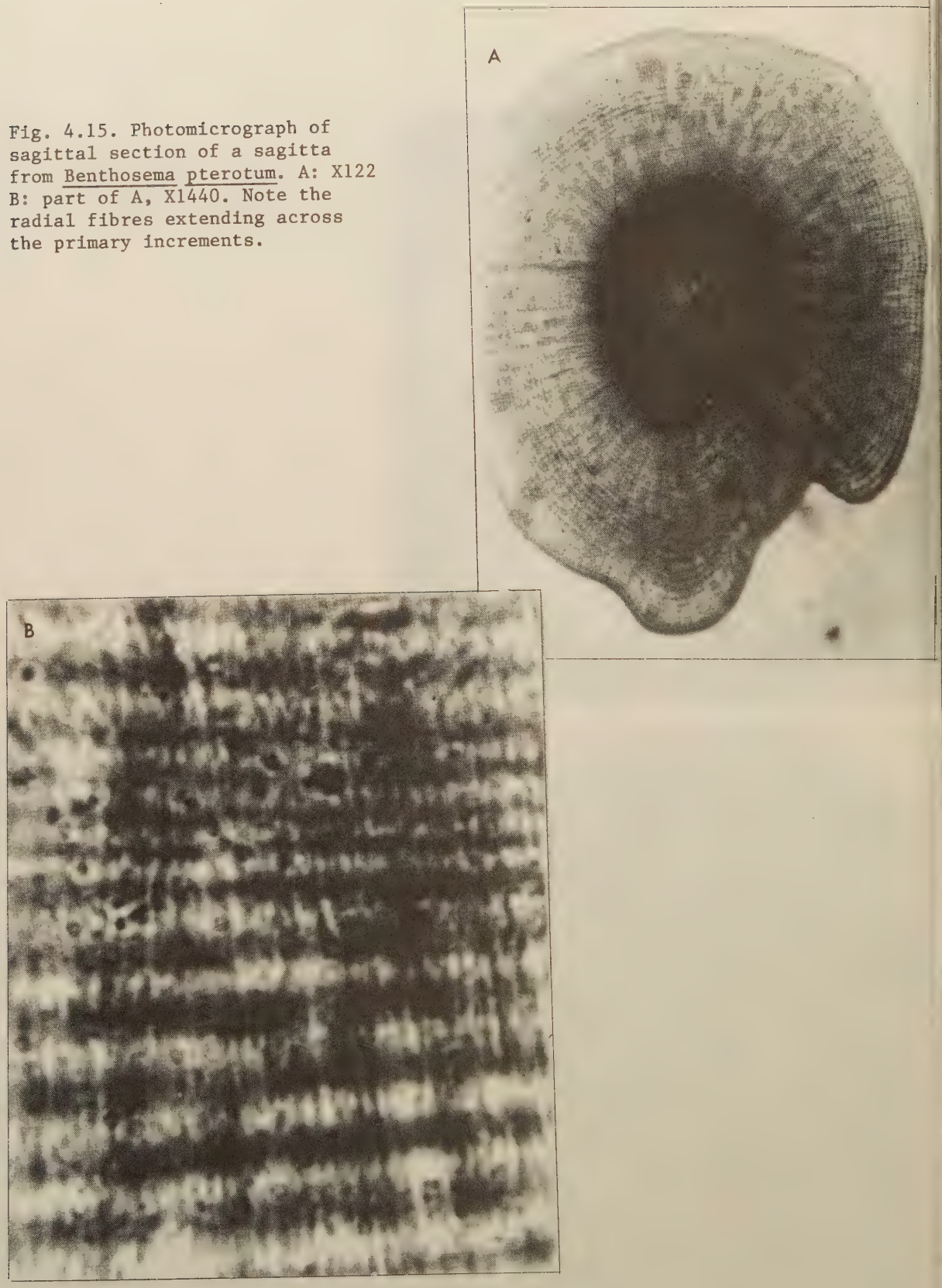


Fig. 4.15. Photomicrograph of sagittal section of a sagitta from Benthosema pterotum. A: X122 B: part of A, X1440. Note the radial fibres extending across the primary increments.

Outside the nuclear area the thickness of the increments varied from 1-2 µm in D. diademophilus to 20 m in S. evermanni.

Verification

Daily periodicity of the primary growth increments in these myctophidae otoliths has not been verified. It seems, however, reasonable to assume such periodicity as a working hypothesis. If these increments are caused by either an internal rhythmicity in the metabolism entrained by outer cyclic stimuli or are caused directly by the periodical/regular change in the external conditions, these factors should probably combine with the diurnal vertical migration to form a daily rhythm.

Growth

Figs. 4.16, 4.17, 4.18 and 4.19 show fish length versus number of growth increments for these species. Assuming that these increments represent daily growth, the figures show length by age. Since little is published about growth rates and life history, the predicted growth curves can only be judged by what seems reasonable. The shape of the curves, i.e. the increase in number of increments with increasing fish length seems plausible. The growth rates indicate a life cycle of about one year for the fastest growing and two or more years for the slow growing species. Predicted hatching times indicate an extended spawning season, which also seems probable.

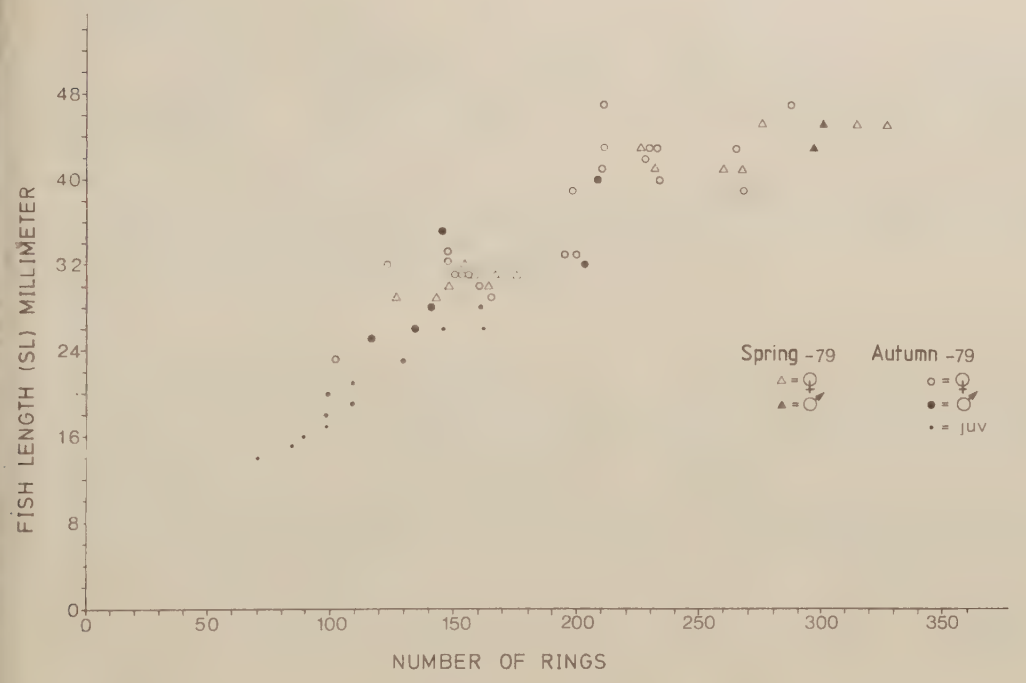


Fig. 4.16. Fish length vs. no. of increments on sagittae for 53 specimens of Benthosema pterotum from the Gulf of Oman 1979.

4.4.5 Dussumieria acuta

The sagittae of 50 specimens of Dussumieria acuta, collected from the East coast of Sri-Lanka were studied. The fish were in the length range 13.0-14.5 cm. The sagittae of all these fishes were more or less of the same size and about 3 mm long. These lengths were read after grinding on a carborundum paper (400-600 grit) in water.

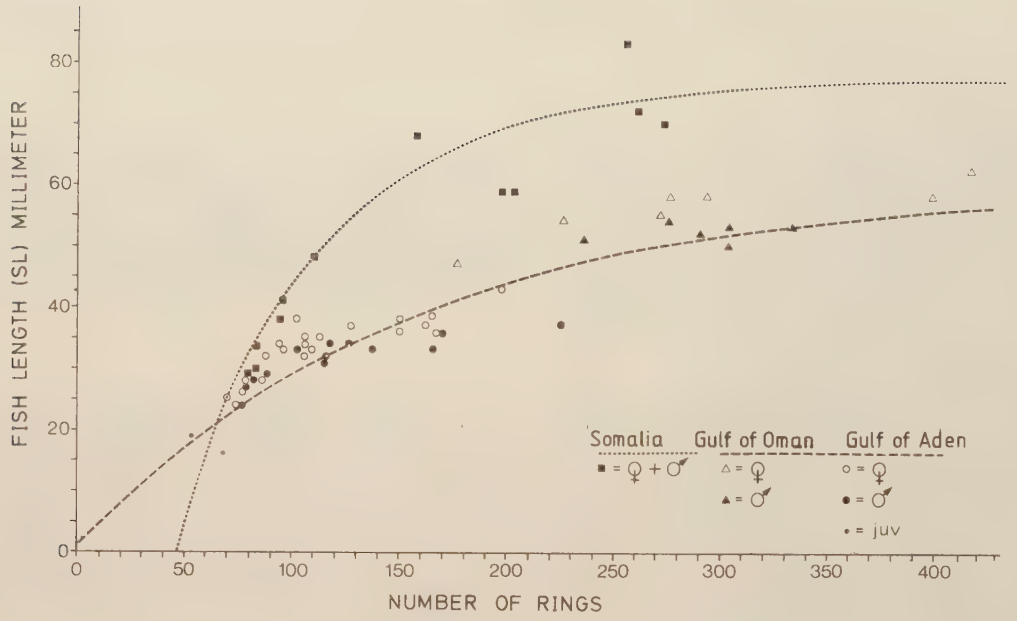


Fig. 4.17. Fish length vs. no. of increments on sagitta. Benthosema fibulatum from Somalia 1975, the Gulf of Aden and the Gulf of Oman 1979. von Bertalanffy growth curves based on the 1975 sample and the 1979 samples respectively are shown.

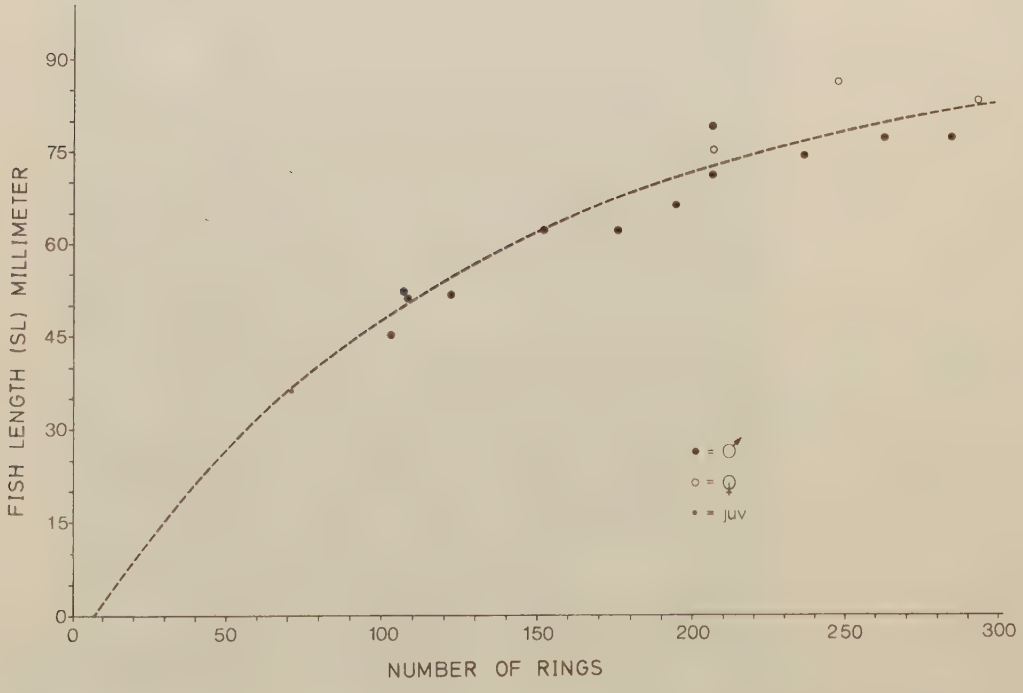


Fig. 4.18. Fish length vs. no. of increments on sagitta for Symbolophorus evermanni.

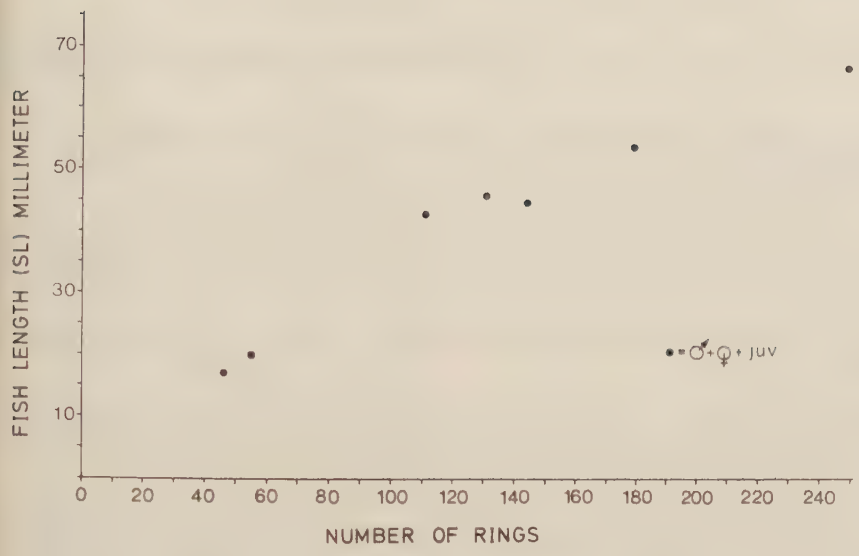


Fig. 4.19. Fish length vs. no. of increments on sagitta for Lampanyctus spp.

Otolith reading

The otoliths were easy to read and could be counted at a lower magnification (x250). The structure as well as the pattern of ring deposition were similar to that of Sardinella species. All the three regions (region A, B and C in Fig. 4.1) differentiated in Sardinella otoliths could be seen in these otoliths too.

Growth

Growth studies were not possible with the available material as they were within a small size range. However, by assuming that these increments represent daily growth, the ring counts indicate that these fish attain a length of 13-14 cm in about one year. No information on growth of this species are available.

The similarity of the otolith microstructure of this species to that of Sardinella spp makes it however, reasonable to consider these primary growth increments as daily.

5. REFERENCES

Bagenal, T. (ed.), 1978 Methods for assessment of fish production in fresh waters. Oxford, Blackwell Scientific Publishers, IBP Handbook, 3rd ed.

Bailey, K., 1982 The early life history of the Pacific hake, Merluccius productus. Fish.Bull.NOAA/NMFS, 80:589-98

Barkman, R.C., 1978 The use of otolith growth rings to age young Atlantic silversides, Menidia menidia. Trans.Am.Fish.Soc., 107:790-2

Barkman, R.C., D.A. Bengtson and A.D. Beck, 1981 Daily growth of the juvenile fish Menidia menidia in the natural habitat compared with juveniles reared in the laboratory. Rapp.P.-V. Reun.CIEM, 178:324-6

Ben-Tuvia, A., 1968 Report on the fisheries investigations on the Israel South Sea Expedition 1962. Bull.Sea Fish.Res.Stn.Haifa, 52:21-55

Bergstad, O.A., 1983 En undersokelse av relasjonen mellom antall vekstsoner i otolittene og alder hos larver og yngel av torsk, Gadus morhua L.. Thesis, University of Bergen, Norway (in Norwegian)

_____, 1984 A relationship between the number of growth increments on the otoliths and age of larval and juvenile cod, Gadus morhua L.. In The propagation of cod, Gadus morhua L., edited by E. Dahl et al. Flodevigen Rapp., 1

Beverton, R.J.H. and S.J. Holt, 1957 On the dynamics of exploited fish populations Fish.Invest.Minist.Agric.Fish.Food G.B.(2Sea Fish.), 19:533 p.

Bingel, F., 1981 Growth of a cod otolith on the crystal level. Meeresforschung/Rep.Mar.Res., 28:212-5

Blacker, R.V., 1975 Stereoscan observations of a plaice otolith. J.Cons.CIEM, 36(2):184-7

Boehlert, G.W., 1981 The effect of photoperiod and temperature on laboratory growth of juvenile Sebastes diploproa and a comparison with growth in the field. Fish.Bull.NOAA/NMFS, 79:789-4

Brothers, E.B., C.P. Mathews and R. Lasker, 1976 Daily growth increments on otoliths from larval and adult fishes. Fish.Bull.NOAA/NMFS, 74:1-8

Brothers, E.B., 1979 Age and growth studies on tropical fishes. In Stock assessment for tropical small-scale fisheries. Proceedings of an International Workshop, September 19-21 1979, edited by S.B. Saila and P.M. Roedel. Kingston, Rhode Island, University of Rhode Island, pp. 119-30

_____, 1981 What can otolith microstructure tells us about daily and subdaily events in the early life history of fish? Rapp.P.-V. Reun.CIEM, 178:393-4

Brothers, E.B. and W.N. Mcfarland, Correlation between otolith microstructure, growth, and life history transitions in newly recruited french grunts (Haemulon flavoleatum (Desmarest), heamulidae). Rapp.P.-V. Reun.CIEM, 178:369-74

Burhanuddin, S.M. and M. Hutomo, 1975 A preliminary study on the growth and food of Stolephorus spp from Jakarta Bay. Mar.Res.Indonesia, 14:1-30

Campana, S.E., 1983 Calcium deposition and otolith check formation during periods of stress in coho salmon, Oncorhynchus kisutch. Comp.Biochem.Physiol.(A Comp.Physiol.), 75:215-20

_____, 1983 Feeding periodicity and the production of daily growth increments in otoliths of steelhead trout (Salmo gairdneri) and starry flounder (Platichtys stellatus). Can.J.Zool., 61:1591-7

Campana, S.E. and J.D. Neilson, Daily growth increments in otoliths of starry flounder (Platichtys
1982 stellatus) and the influence of some environmental variables in their production. Can.J.Fish.Aquat.Sci., 39(7):937-42

Cochran, W.G., Sampling techniques. New York, J.Wiley and Sons, Inc. 2nd ed.
1963

Dale, T., Embryogenesis of the otolith organs of the cod Gadus morhua L. (Teleostei: Gadidae) with
1984 special reference to the formation and growth of otoliths. In The propagation of cod Gadus morhua L. edited by E. Dahl, et al.. Flodevigen Rapp., 1

Dayaratne, P., Age and growth studies of four Sardinella spp. from Sri-Lanka by using primary
1983 growth rings in otoliths. Thesis. University of Bergen, Norway

Degens, E.T., W.G. Deuser and R.L. Haedrich, Molecular structure and composition of fish otoliths.
1969 Mar.Biol., 2(2):105-13

Dunkelberger, D.G., J.M. Dean and N. Watabe, The ultrastructure of the otolith membrane and
1980 otolith in the juvenile mummichog, Fundulus heteroclitus. J. Morphol., 163:367-77

Fagade, S.O., The structure of the otoliths of Tilapia guineensis (Dumeril) and their use in age
1979 determination. Hydrobiologia, 69:169-73

_____, The morphology of the otoliths of the Bagrid catfish Chrysichthys nigrodigitatus
1980 (Lacepede) and their use in age determination. Hydrobiologia, 71:209-15

Geffen, A.J., Otolith ring deposition in relation to growth rate in herring (Clupea harengus) and
1983 turbot (Scophthalmus maximus) larvae. Mar.Biol., 71:317-26

Gjosaeter, H., Dagsonelesing som metode i aldersstudie pa fish, med anvendelse pa tropiske og
1981 boreale arter. Thesis. University of Bergen, Norway (in Norwegian)

Gjosaeter, H. and V. Oiestad, Growth patterns in otoliths as an indication of daily growth variations
1981 of larval herring (Clupea harengus) from an experimental ecosystem. ICES CM 1981 H:31:7 p. (mimeo)

Gjosaeter, H. and S. Tilseth, Primary growth increments in Otoliths of cod larvae (Gadus morhua L.)
1982 of the Arcto-Norwegian cod stock. Fiskeridir.Skr.(Havunders.) 17:287-95

Gjosaeter, J., Aspects of the distribution and ecology of the Myctophidae from the western and
1978 northern Arabian Sea. Rome, FAO/UNDP, Norway Funds-in-Trust, Indian Ocean Programme Development Report, (43)Vol.2:2:62-108

_____, Life history and ecology of Maurolicus Muelleri (Gonostomatidae) in Norwegian
1981a waters. Fiskeridir.Skr.(Havunders) 17:133-52

_____, Abundance and production of lanternfish (Myctophidae) in the western and northern
1981b Arabian Sea. Fiskeridir.Skr.(Havunders), 17:215-51

Gjosaeter, J., I.M. Beck and T. Monstad, Primary growth rings in Blue Whiting otoliths. ICES CM
1979 1979:12 p. (mimeo)

Gjosaeter, J. and T. Monstad, Primary growth rings in otoliths of the Barents Sea capelin. ICES CM
1982 1982 (H:9) (mimeo)

Gjosaeter, J. and M.I. Sousa, Reproduction, age and growth of the kelee shad, Hilsa kelee (Cuvier,
1983 1829) (Pisces: Fam. Clupeidae) with information on its fishery in Maputo Bay, Mozambique. Rev.Invest.Pesq., Maputo, 8:53-80

_____, Reproduction, age and growth of the Russell scad, Decapterus russellii (Ruppel, 1828)
1983a (Carangidae) from Sofala Bank, Mozambique. Rev.Invest.Pesq., Maputo, 8:83-104

Grice, G.D. and M.R. Reeve (eds), Marine mesocosms. Biological and chemical research in
1982 experimental ecosystems. New York, Springer-Verlag

Hay, D.E., 1982 Fixation shrinkage of herring larvae: effects of salinity, formation concentration and other factors. Can.J.Fish.Aquat.Sci., 39:1138-43

Kendall, A.W.Jr. and D. Gordon, 1981 Growth rate of Atlantic mackerel (Scomber scombrus) larvae in the middle Atlantic Bight. Rapp.P.-V.Reun.CIEM, 178:337-41

Kochin, P.N., 1982 Study of age and growth of pike glassfish, Champsocephalus gunnari (Channichtyidae). J.Ichthyol., 22(3):37-47

Krishnamoorthi, B., 1976 A note on the size difference between males and females of Namipterus japonicus (Block). Indian J.Fish., 21(2):608-9

Laroche, J.L., S.L. Richardson and A.A. Rosenberg, 1982 Age and growth of a pleuronectid, Parophrys vetulus, during the pelagic larval period in Oregon coastal waters. Fish.Bull. NOAA/NMFS, 80(1):93-104

Le Guen, J.C., 1976 Utilisation des otolithes pour la lecture de l'age de sciaenides intertropicaux margues saisonnieres et journalieres. 1. Cah.ORSTOM (Oceanogr.), 14(4):331-8

Liew, P.K.L., 1974 Age determination of American eels based on the structure of their otoliths. The ageing of fish, edited by T.B. Bagenal. Old Working, Surrey, Unwin Brothers Ltd., pp. 124-36

Lissa, J.van, 1982 Growth of larval Engraulicypris sardella as determined by daily growth increments on the Astericus, Lapillus and Sagitta otoliths. Fishery expansion project, Malawi. Biological studies on the pelagic ecosystem of Lake Malawi. Rome, FAO/UNDP. Technical report, 1:51-6

Laugh, R.G., 1982 Age and growth of larval Atlantic herring, Clupea harengus L., in the Gulf of Maine - Georges Bank regions based on otolith growth increments. Fish.Bull.NOAA/NMFS, 80:187-99

Marshall, L.S. and S.S. Parker, 1982 Pattern identification in the microstructure of sockeye salmon (Oncorhynchus nerka) otoliths. Can.J.Fish.Aquat.Sci., 39:542-7

Mendiola, R. and C.O. Gomez, 1981 Daily rings in otoliths of larval anchovy (Engraulis ringens J.). Rapp.P.-V. Reun.CIEM, 178:565-6

Methot, R.D.Jr., 1981 Spatial covariation of daily growth rates of larval northern anchovy Engraulis mordax and northern lampfish Stenobrachius leucopsarus. Rapp.P.-V.Reun.CIEM, 178:424-31

Methot, R.D. Jr. and D. Kramer, 1979 Growth of northern anchovy, Engraulis mordax, larvae in the sea. Fish.Bull.NOAA/NMFS, 77:413-23

Miranda, O.B., 1981 Daily lines of growth as components of the spawning bands in the otoliths in a tropical fish (Saprisoma aurofrenatum) (Cuvier and Valenciennes, 1839). Rev.Biol.Mar.Inst. Oceanol.Univ.Valparaiso, 17(2):253-65

Morales-Nin, B., 1980 Incrementos de crecimiento diario en las sagittas de Merluccius paradoxus Franca. Invest.Pesq.Barc., 44:305-12

Mugiya, Y., et al., 1981 Diurnal rhythm in otolith formation in the gold fish, Carassius auratus. Comp.Biochem.Physio.(A Comp.Physiol.) 68:659-62

Muller, R.G., 1977 Some aspects of the population biology of Stolephorus heterolobus from Palau. NOAA Tech.Rep.NMFS Circ., (408):119-26

Neilson, J.D. and G.H. Geen, 1981 Methods for preparing otoliths for microstructure examination. Prog.Fish-Cult., 43:90-1

_____, 1982 Otoliths of chinook salmon (Onchorynchus tshawytscha): daily growth increments and factors influencing their production. Can.J.Fish.Aquat.Sci., 39(10):1340-7

Pannella, G., 1971 The otoliths: daily growth layers and periodical patterns. Science,Wash., 173:1124-7

_____, Otolith growth patterns: an aid in age determination in temperate and tropical
1974 waters. In The ageing of fish, edited by T.B. Bagenal. Old Woking, Surrey Unwin Brothers Ltd., pp. 28-39

_____, Growth patterns in fish sagittae. In Skeletal growth in aquatic organisms: biological
1980 records of environmental change, edited by D.C. Rhoads and R.A. Lutz. New York, Plenum Press, pp. 519-60

Pannella, G., Methods of preparing fish sagittae for the study of growth patterns. In Skeletal growth
1980 in aquatic organisms: biological records of environmental change, edited by D.C. Rhoads and R.A. Lutz. New York, Plenum Press, pp. 613-23

Parrish, J.D., Approaches to some problem areas in tropical small-scale fisheries In Stock assessment
1979 for tropical small-scale fisheries. Proceedings of an International Workshop, September 19-21, 1979, edited by S.B. Saila and P.M. Roedel. Kingston, Rhode Island, University of Rhode Island.

Pauly, D. and N. David, ELEFAN 1, a BASIC Program for the objective extraction of growth
1981 Parameters from length-frequency data. Meeresforschung/Rep.Mar.Res., 28:205-11

Penney, R.W. and J.J. Anderson, Otolith analysis of age and growth of larval redfish (Sebastes sp.) on
1981 Flemish Cap. 1979 and 1980. NAFO Scr.Doc., 81/IX/117. Serial No. N423,

Radtke, R.L., The formation and growth of otoliths from redfish (Sebastes spp.) on Flemish Cap,,
1980 1979 and 1980. NAFO Scr.Doc. 80/19 153, Serial No. N225

Radtke, R.L., M. Collins and J.M. Dean, Morphology of the otoliths of the Atlantic blue marlin
1982 (Makaira nigricans) and their possible use in age estimation. Bull.Mar.Scie., 32:498-503

Radtke, R.L. and J.M. Dean, Increment formation in the otoliths of embryos, larvae and juveniles of
1982 the mummichog, Fundulus heteroclitus. Fish.Bull.NOAA/NMFS, 80(2):201-15

Radtke, R.L. and K.G. Waiwood, Otolith formation and body shrinkage due to fixation in larval cod
1980 (Gadus morhus). Can.Tech.Rep.Fish.Aquat.Sci., (929):10 p.

Ralston, S., Age determination of a tropical reef butterflyfish utilizing daily growth rings of otoliths.
1976 Fish.Bull.NOAA/NMFS, 74:990-4

Rannou, M. and C. Thiriot-Quievreux, Structure des otoliths d'un Macrouridae (Poisson Gadiforme)
1975 bathyal. Edtude au microscope electronique a balayage. Ann.Inst.Oceanogr.,Paris, 51(2):195-201

Re, P., Daily growth increments in the sagitta of pilchard larvae Sardina pilchardus (Walbaum, 1972)
1983 Pisces: Clupeidae). Cybium, 7(3):9-15

Rosenberg, A.A., Growth of juvenile English sole, Parophrys vetulus, in estaurine and open coastal
1982 nursery grounds. Fish.Bull.NOAA/NMFS, 80(2):245-52

Rosenberg, A.A. and A.S. Haugen, Individual growth and size-selective mortality of larval turbot
1982 (Scophthalmus maximus) reared in enclosures. Mar.Biol., 72:73-7

Rosenberg, A.A. and J.L. Laroche, Growth during metamorphosis of English sole, Parophrys vetulus.
1982 Fish.Bull.NOAA/NMFS, 80(2):150-3

Rosenberg, A.A. and G.R. Lough, A preliminary report on the age and growth of larval herring
1977 (Clupea harengus) from daily growth increments in otoliths. ICES CM 1977 L:20:16 p. (mimeo)

Schmidt, P.E. and M.C. Fabrizio, Daily growth rings on otoliths for ageing young of the year
1980 largemouth bass from a wild population. Prog.Fish-Cult., 42:78-80

Sokal, R.S. and F.J. Rohlf, Biometry. San Francisco W.H. Freeman and Co.
1969

Steffensen, E., 1980 Daily growth increments observed in otoliths from juvenile East Baltic cod. Dana, 1:29-37

Struhsaker, P. and J.H. Uchiyama, 1976 Age and growth of the Nehu, Stolephorus purpureus (Pisces: Engraulidae), from the Hawaiian islands as indicated by daily growth increment of sagittae. Fish.Bull.NOAA/NMFS, 74:9-16

Tanaka, K., Y. Mugiya and I. Yamada, 1981 Effects of photoperiod and feeding on daily growth patterns in otoliths of juvenile Tilapia nilotica. Fish.Bull.NOAA/NMFS, 79:459-66

Taubert, B.D. and D.W. Coble, 1977 Daily rings in otoliths of three species of Lepomis and Tilapia mossambica. J.Fish.Res.Board Can., 34:332-40

Tham, A.K., 1966 A contribution to the study of the growth of members of the genus Stolephorus Lacepede in Singapore Straits. Proc.IPFC, 12(2):1-25

Theilackerer, G.H., 1980 Changes in the body measurements of larval northern anochovy, Engraulis mordax, and other fishes due to handling and preservation. Fish.Bull.NOAA/NMFS, 78(3):685-92

Timola, O., 1977 Scanning electron microscope studies on the growth patterns of smelt, Osmerus eperlanus (L.) otoliths. Aquilo (Zool.) 17:57-60

Tiews, K., I.A. Ronquillo and P. Cases-Borja, 1970 On the biology of roundscads (Decapterus Bleeker) in the Philippine waters. Proc.IPFC, 13:82-106

Townsend, D.W., 1980 Microstructural growth increments in some Antarctic fish otoliths. Cybium (3e Ser.), 1980(8):17-22

Townsend, D.W. and J.J. Graham, 1981 Growth and age structure of larval Atlantic herring, Clupea harengus harengus, in the Sheepscot River estuary, as determined by daily growth increment in otoliths. Fish.Bull.NOAA/NMFS, 79:123-30

Townsend, D.W. and R.F. Shaw, 1982 Daily growth increments in otoliths of blue whiting, Micromesistius poutassou (Risso) from above the Arctic Circle. Sarsia, 67:143-7

Uchiyama, J.H. and P. Struhsaker, 1981 Age and growth of skipjack tuna Katsuwonus pelamis and yellowfin tuna Thunnus albacares as indicated by daily growth increments of sagittae. Fish.Bull.NOAA/NMFS, 79:151-62

Victor, B.C., 1982 Daily otolith increments and recruitment in two coral-reef wrasses, Thalassoma bifasciatum and Halichoeres bivittatus. Mar.Biol., 71:203-8

Victor, B.C. and E.G. Brothers, 1982 Age and growth of the fallfish, Semotilus corporalis with daily otolith increments as a method of annulus verification. Can.J.Zool., 60:2543-50

Weber, W. and A.A. Jothy, 1977 Observations on the fish Nemipterus spp. (Family: Nemipteridae) in the coastal waters of East Malaysia. Arch.Fischereiures., 28:109-22

Wild, A. and T.J. Foreman, 1980 The relationship between otolith increments and time for yellowfin and skipjack tuna, marked with tetracycline. Bull.I-ATTC, 17:509-60

Wilson, K.H. and P.A. Larkin, 1980 Daily growth rings in the otoliths of juvenile sockeye salmon (Oncorhynchus nerka). Can.J.Fish.Aquat.Sci., 37:1495-8

Wilson, K.H. and P.A. Larkin, 1982 Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (Oncorhynchus nerka) Can.J.Fish.Aquat. Sci., 39:1335-9

Worthmann, H., 1980 The early growth stages of the pescada (Plagioscion monti Soares). Arch.Fischereiwiss., 3:145-56

Yanez-Arancibia, A. and V.L. de Yanez, 1977 Desarrollo del otolito embrionario, patron de su crecimiento y comparacion morfologica con otolitos juveniles y adultos del bagre marno Galeichthys caerulescens (Gunther). (Development of the embryonic otolith, pattern of its growth and morphological comparison with otolith of juvenile and adult sea catfish (Galeichthys caerulescens). Ann.Cent.Cienc.Mar.Limnol.Univ.Nac.Auton.Mex., 4(1):115-24